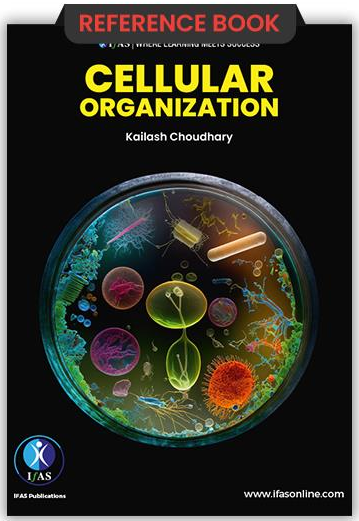
Don't wait for the opportunity. Create it.

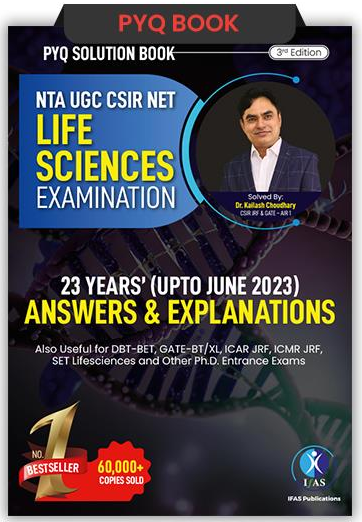
CSIR NET – Life Science

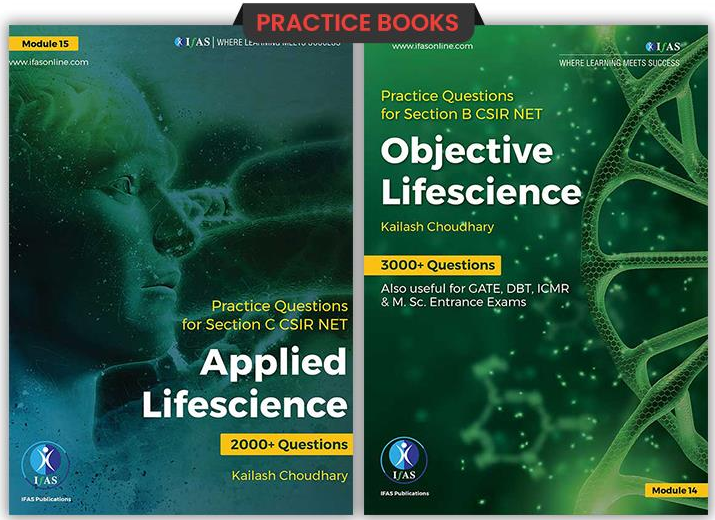
Unit 2: Cellular Organization

L3

Plasma Membrane proteins




PYQ BOOK
PYQ SOLUTION BOOK
3rd Edition
NTA UGC CSIR NET
LIFE SCIENCES
EXAMINATION
23 YEARS' (UPTO JUNE 2023)
ANSWERS & EXPLANATIONS
Also Useful for DBT-BET, GATE-BT/XL, ICAR JRF, ICMR JRF, SET Lifesciences and Other Ph.D. Entrance Exams
NO 1 BESTSELLER
60,000+ COPIES SOLD

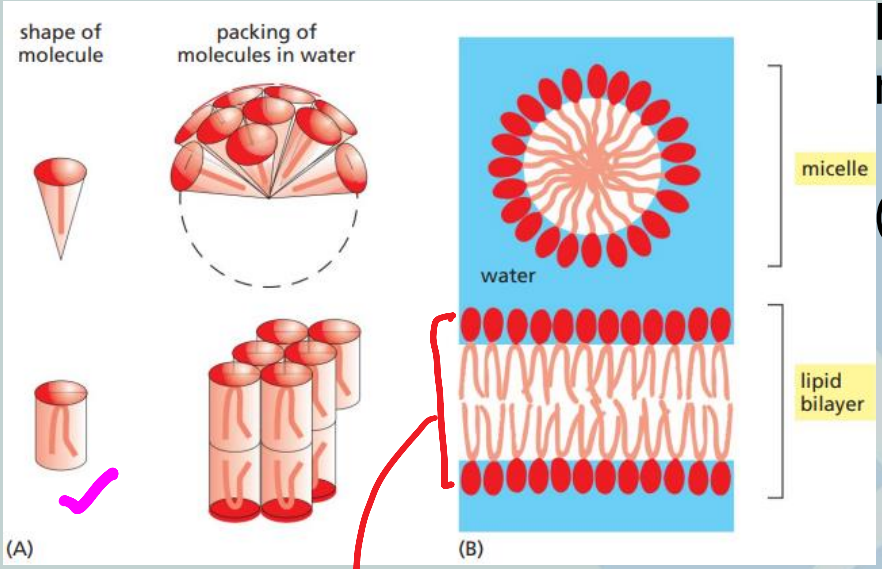


IfAS

Order Online and Get Free Delivery Across India

Points to be covered in this Lecture

- Archie Lipids, Liposomes and micelles
- Structure of cholesterol, lipid rafts and and caveolae
- Plasma Membrane-proteins
- Isolation of protein
- Hydropathy plot
- Cell fusion expo, FRAP

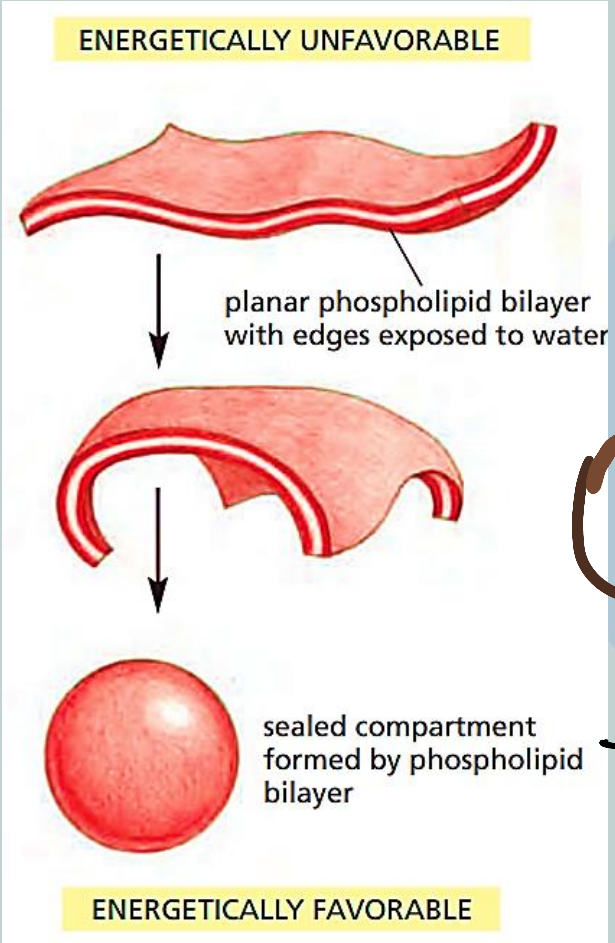


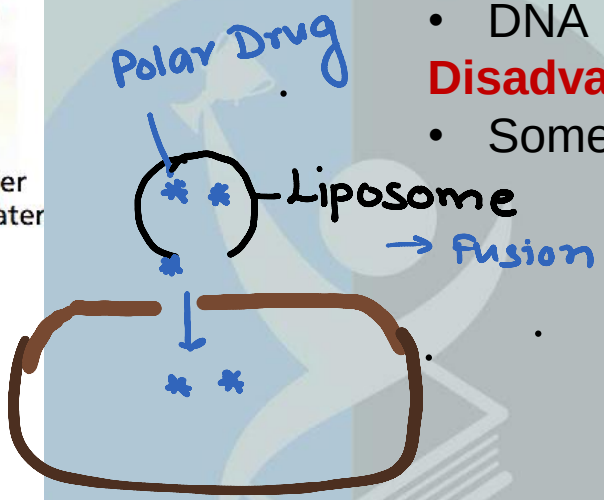
major interaction
- Hydrophobic
- H- Bonding
- Vonder-wall

Packing arrangements of amphiphilic molecules in an aqueous environment.

(A) These molecules spontaneously form micelles or bilayers in water, depending on their shape. Cone-shaped amphiphilic molecules (above) form micelles, whereas cylinder-shaped amphiphilic molecules such as phospholipids (below) form bilayers.

(B) A micelle and a lipid bilayer seen in cross section.


ENERGETICALLY UNFAVORABLE
planar phospholipid bilayer with edges exposed to water
sealed compartment formed by phospholipid bilayer
ENERGETICALLY FAVORABLE



known as → Liposome

Application of Liposome

- Drug delivery
- DNA Delivery

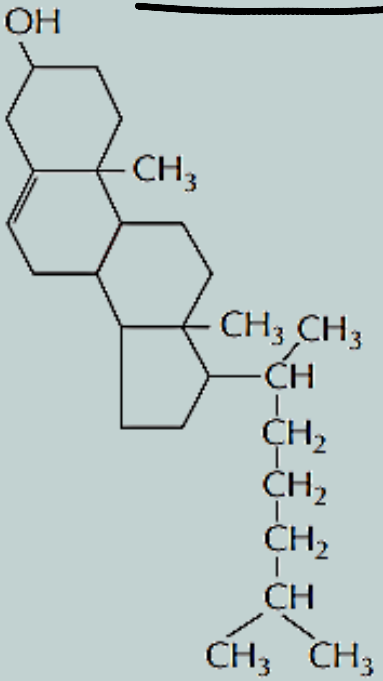
Disadvantage

- Some times undergoes phagocytosis

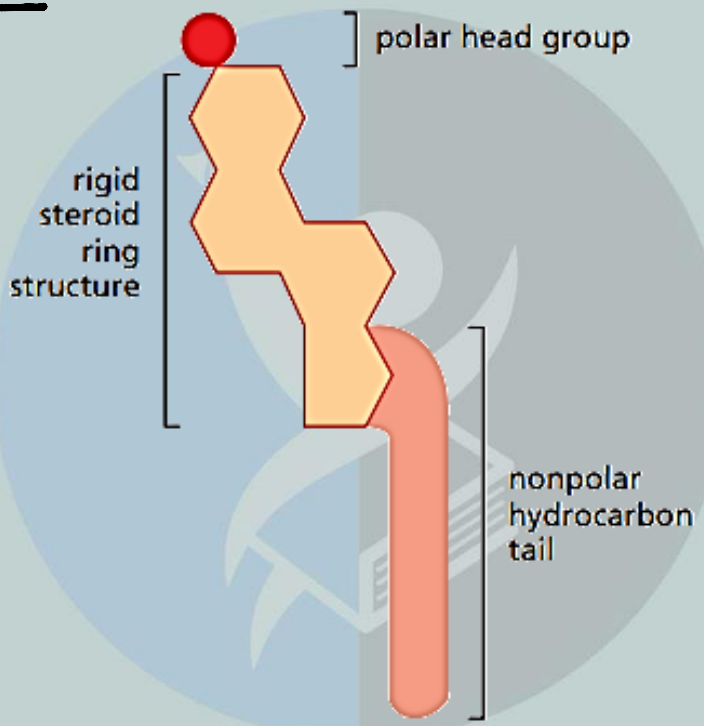
* Disadvantage
- Some time undergo Phagocytosis

- Lipid bag

Cholesterol



(A)

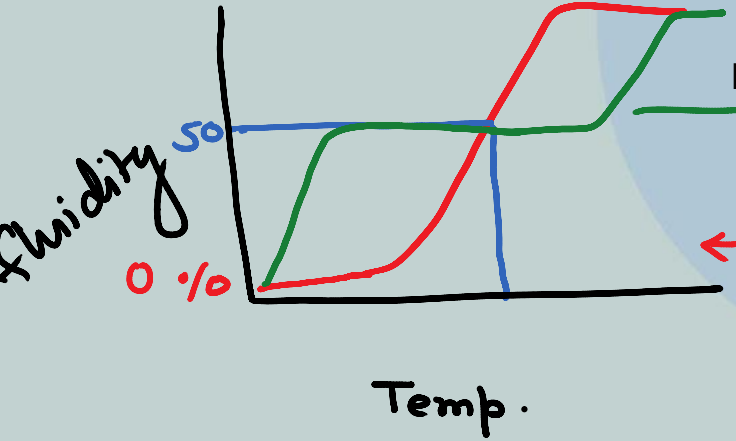


(B)

(C)

Role of Cholesterol?

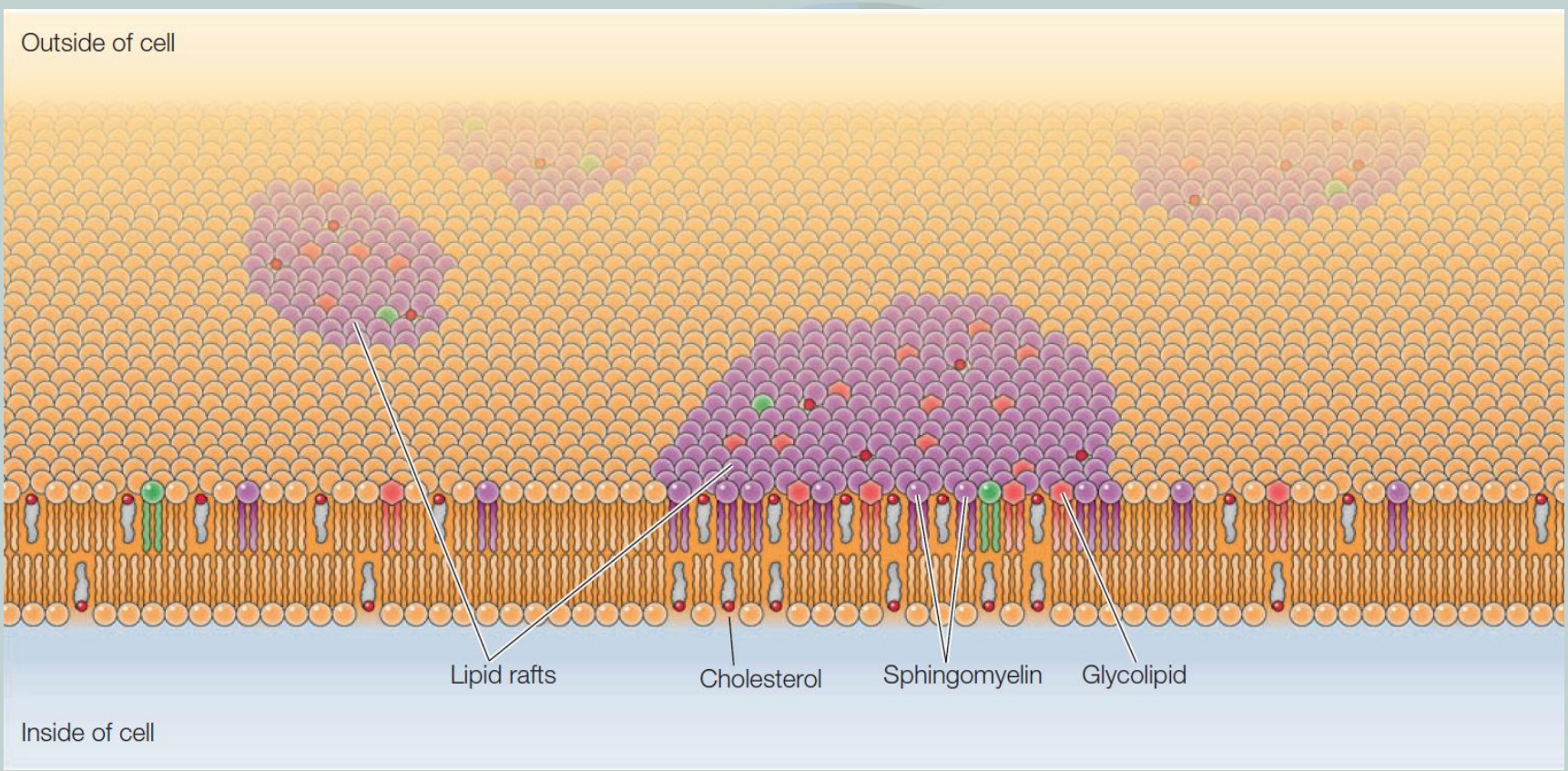
- Provide rigidity to plasma membrane
- Maintain fluidity of plasma membrane
- Limit the movement of lipid in bilayer



Fluidity in presence of cholesterol

- Act as temp. buffer
- Cholesterol do not affect Tm

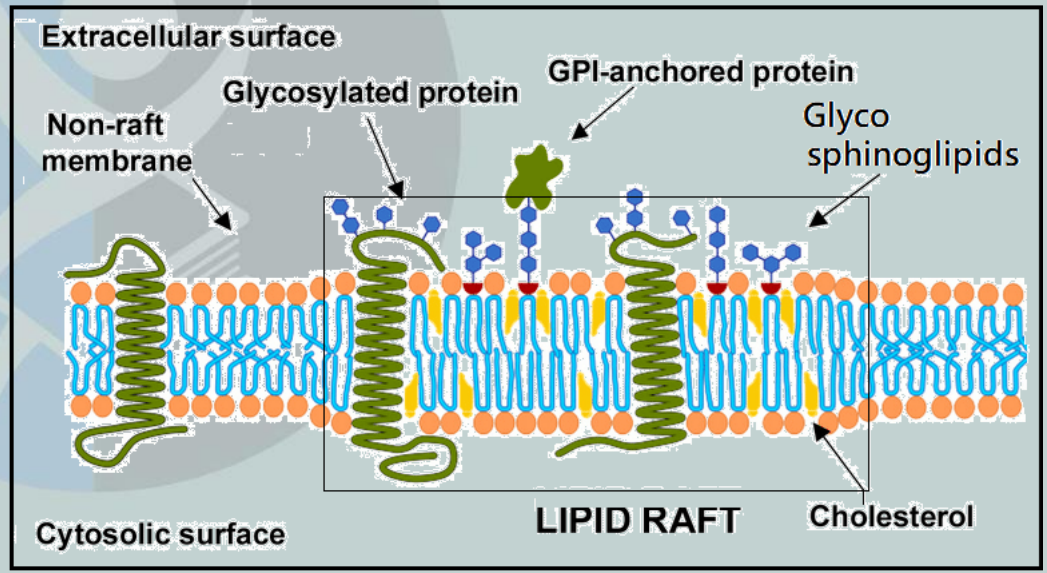
Lipid Rafts Sphingolipids and cholesterol



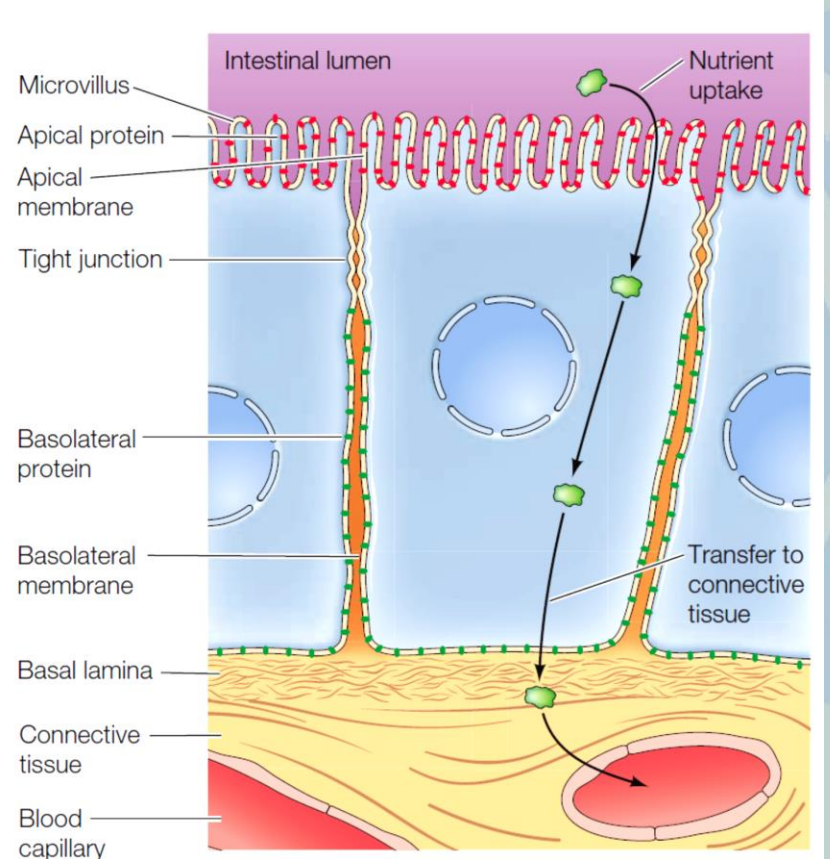
Lipid rafts

The planar cholesterol molecules are postulated to fill the voids that form underneath the large head groups of the sphingolipids

- Lipid rafts are localized regions of membrane lipids that are involved in cell signaling
- It contains sphingolipids, cholesterol and various proteins.

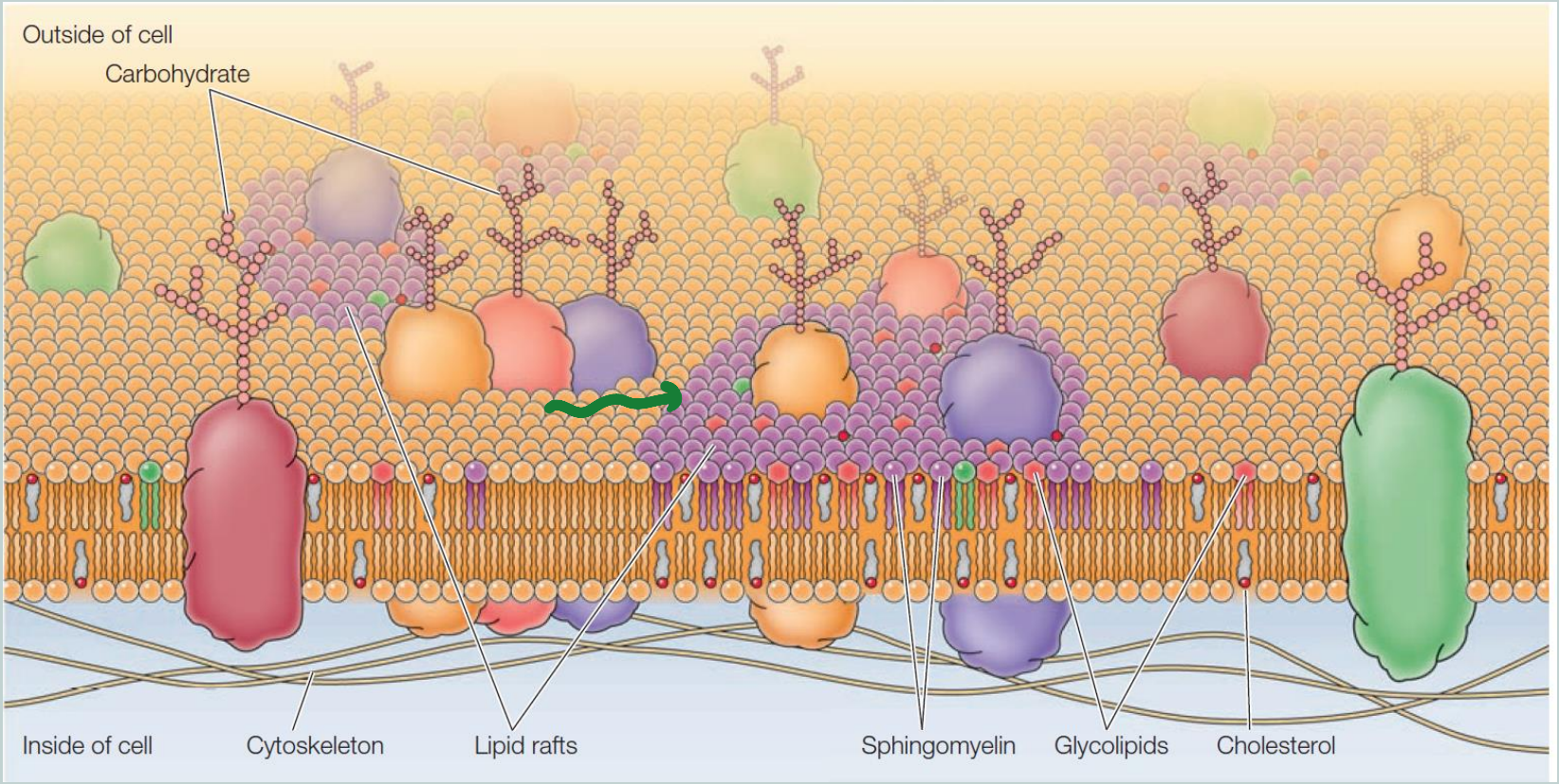


Proteins are restricted to the apical or basolateral domains of epithelial cells.



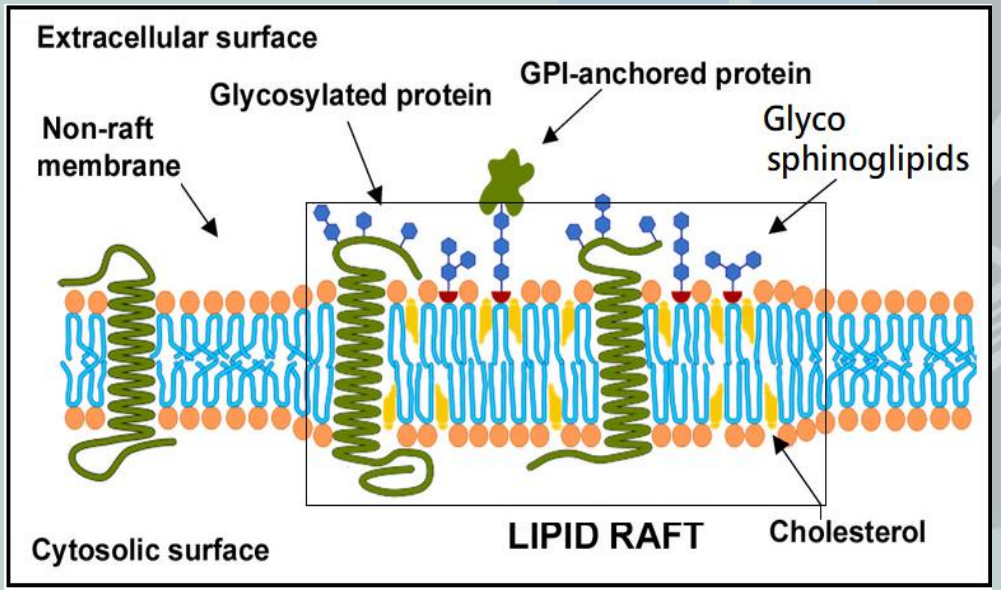
- **Raft** – Kept important protein in aggregation (Receptors)
- Limit the movement of proteins

Lipid rafts - clustered domains: Sphingomyelin, glycolipids, cholesterol



Lipid Rafts

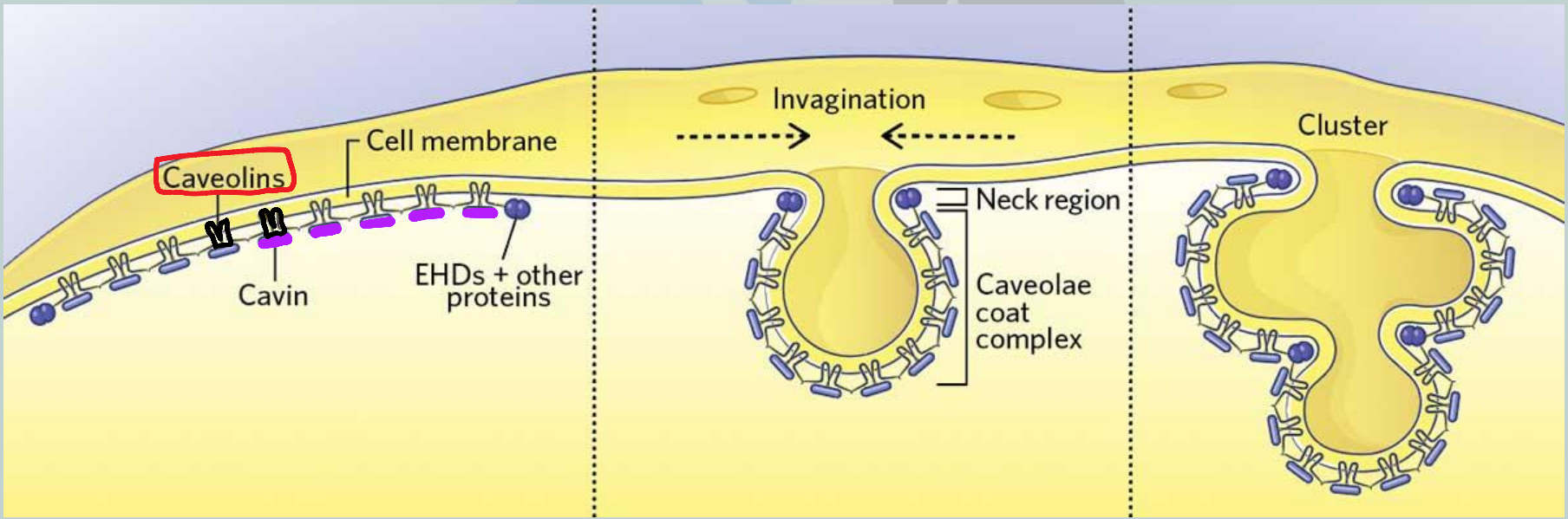
- Lipid rafts are localized regions of membrane lipids that are involved in cell signaling
- It contains sphingolipids, cholesterol and various proteins.



Caveolae (“little caves”): Subset of lipid rafts , 60- to 80-nm invaginations
Membrane protein caveolin, interacts with the cytoplasmic protein cavin

- Caveolin also interacts with cholesterol
- Endothelial cells and Adipocytes.

Functions: Endocytosis, cell signaling, regulation of lipid transport and protection of the plasma membrane against mechanical stress.



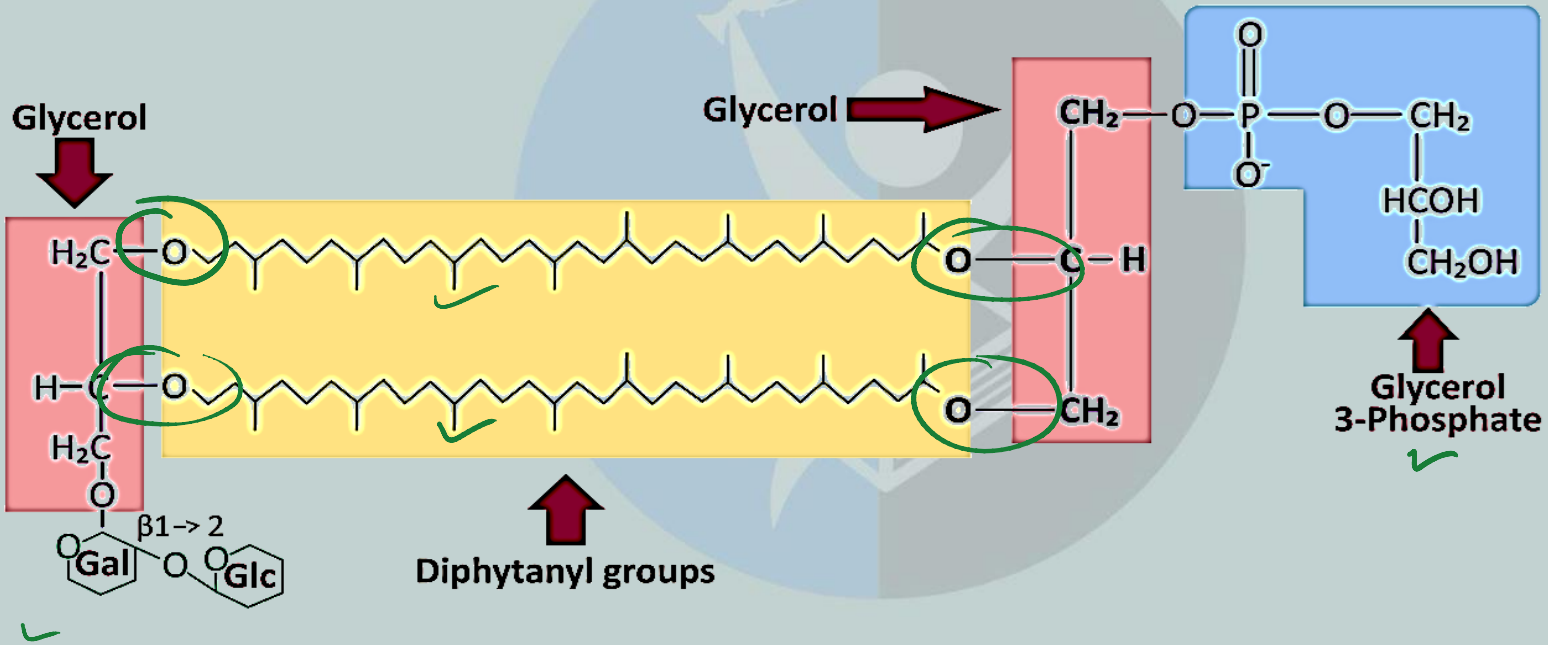
Phospholipid structures in Archaea

L-glycerol
Ether bonds [strong]
Branch chain hydrocarbons

→ Can lives in Extreem Environment

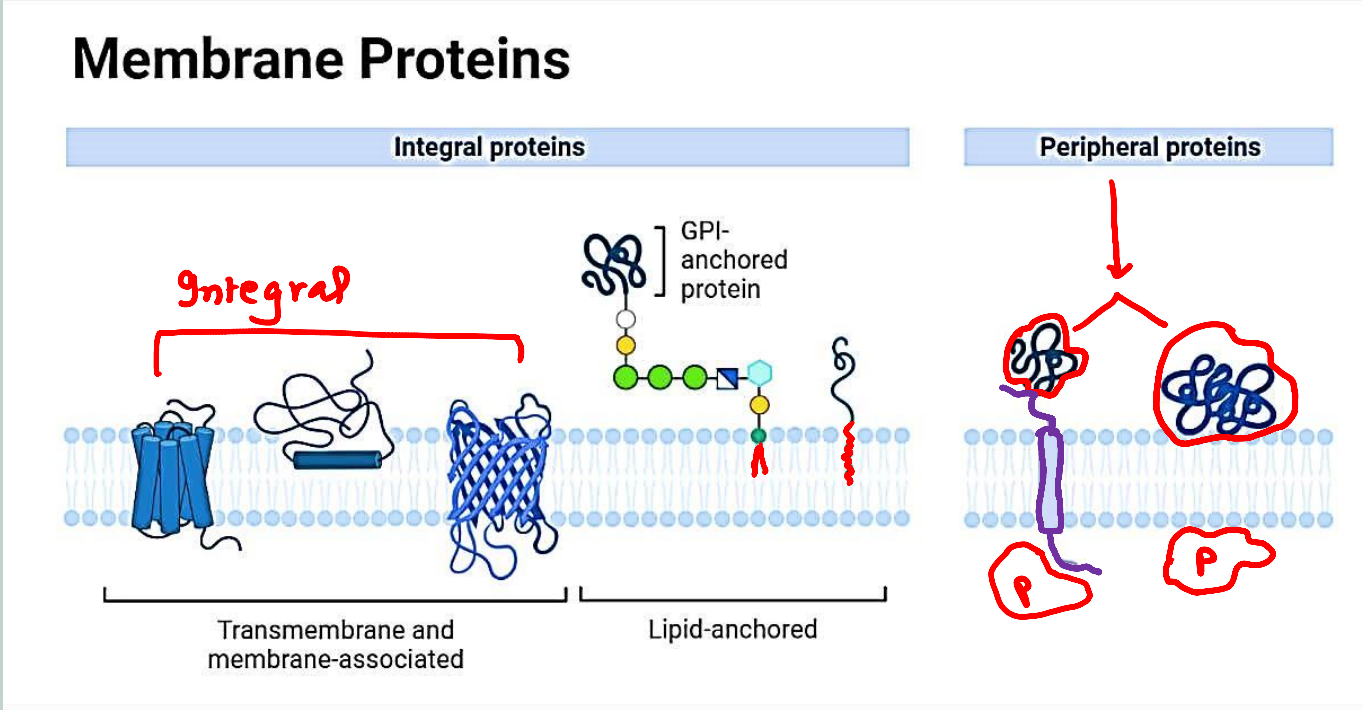
Glycerol dialkyl glycerol tetraethers (GDGTs)

Isoprenoid chains



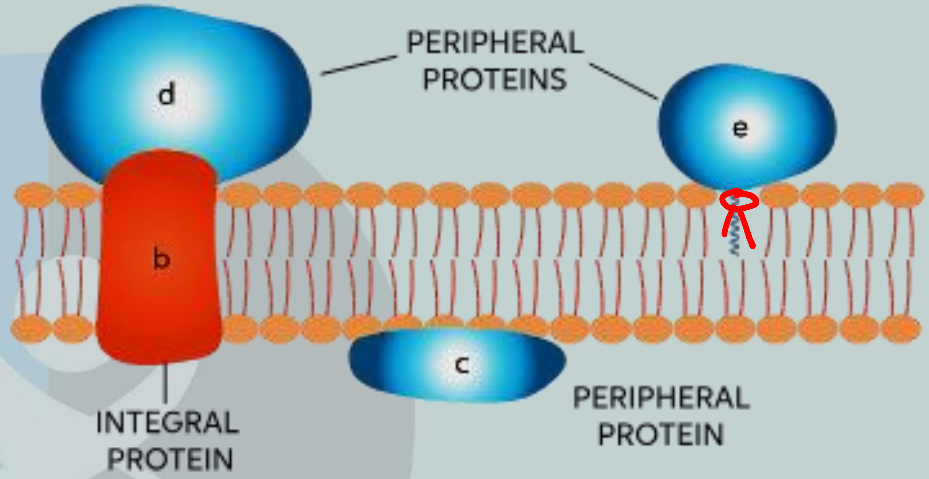
Membrane Proteins

- Peripheral
- Integral
- Lipid Anchored



Peripheral proteins

- Present on the surface of plasma membrane
- Exoplasmic peripheral ✓
- Cytoplasmic peripheral ✓

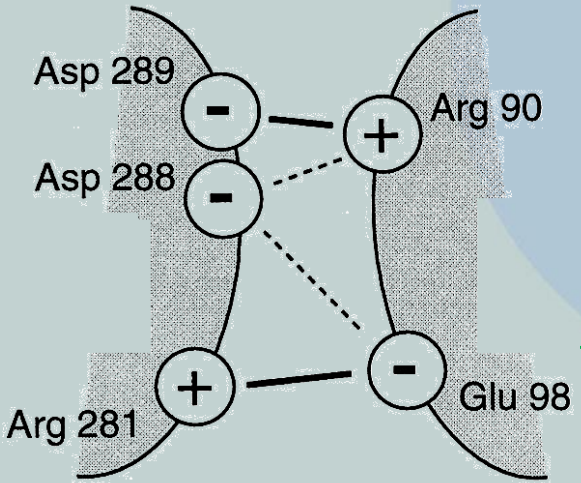


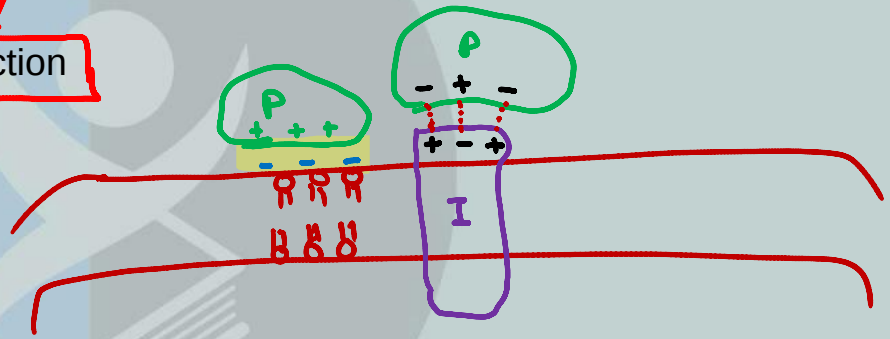
- Globular in nature → core Hydrophobic
  Surface Hydrophillic

Interactions

- **Peripheral proteins:** Globular proteins interact with the membrane mainly through electrostatic and hydrogen-bonding interactions

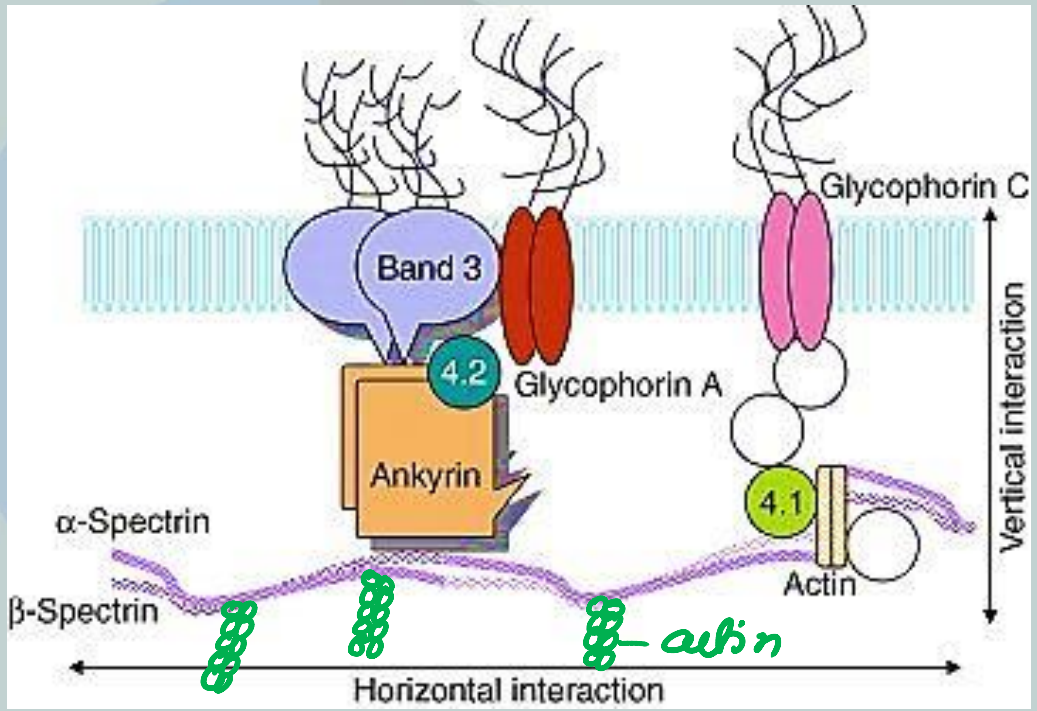
Weak interaction





Examples of Peripheral Protein on cytosolic face of RBC

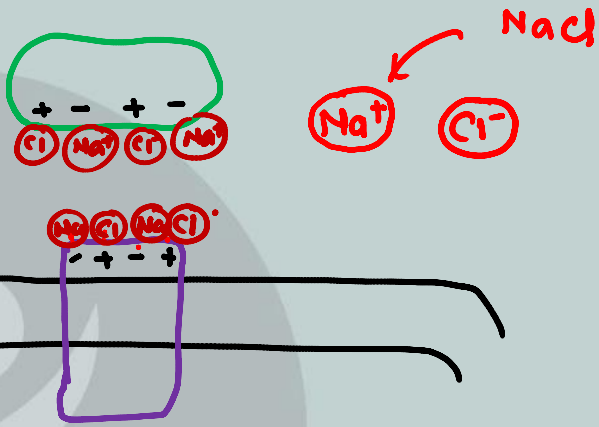
Spectrin
Actin,
Ankyrin
Band 4.1



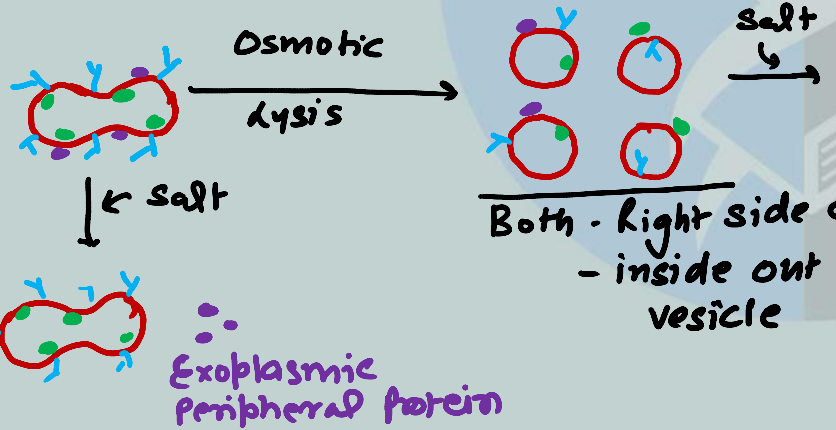
Separation of Peripheral Protein

- extreme pH (5) or high salt (1.5M NaCl)

* PH can Alter the charge on a.a
- Can break interaction

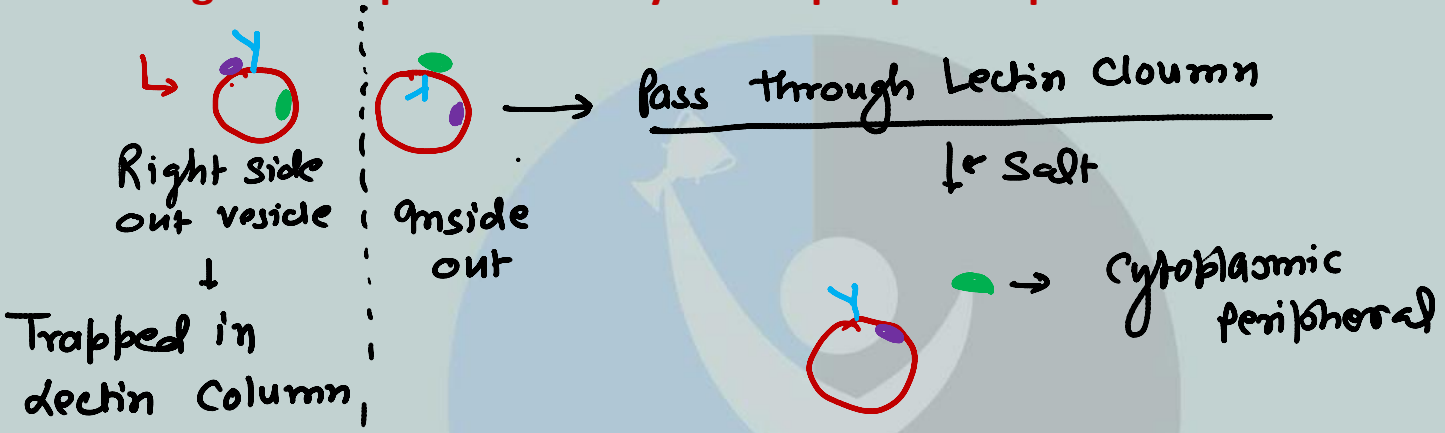


Que

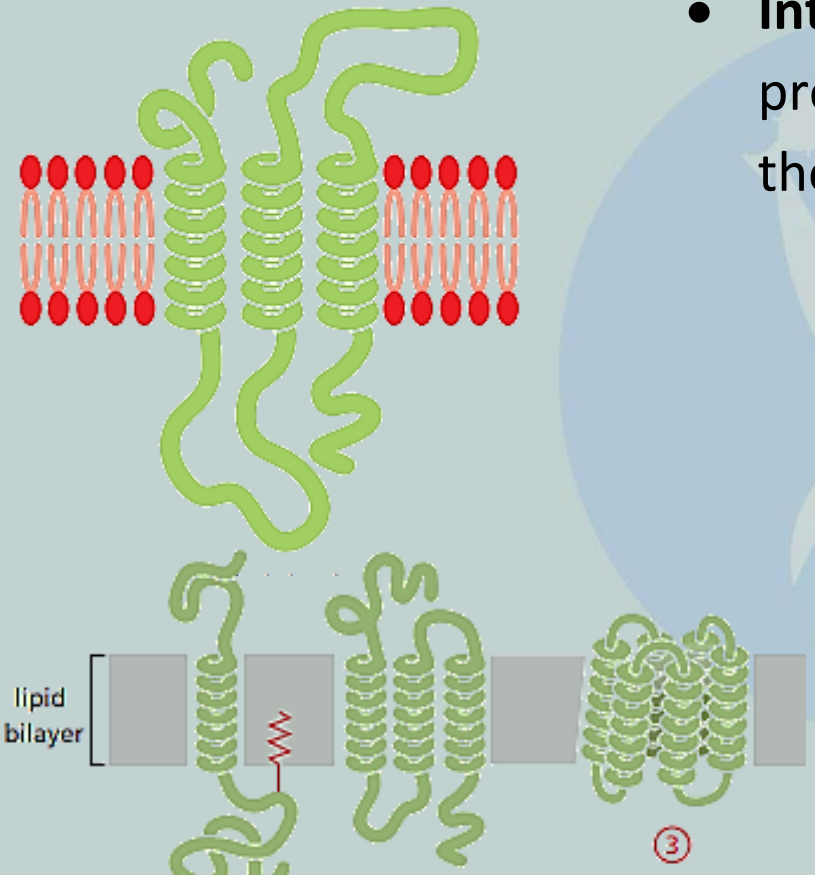


Both · Cytoplasmic Peripheral
· Exoplasmic n
] Separate

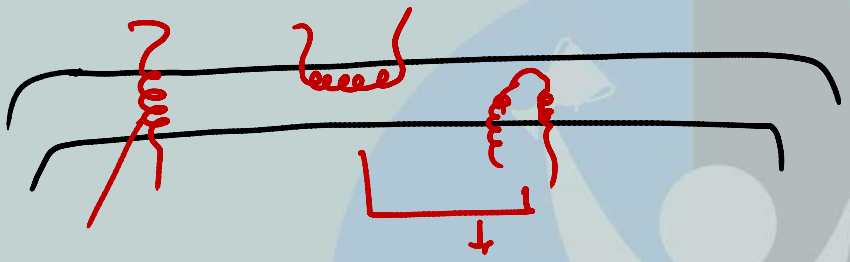
How to distinguish exoplasmic and cytosolic peripheral protein?



Integral Membrane Protein



- **Integral proteins**: A portion of the protein embedded in, or extending all the way across, the lipid bilayer.



Trans membrane

↓

Span at least once to the Plasma membrane.

Integral but not Transmembrane

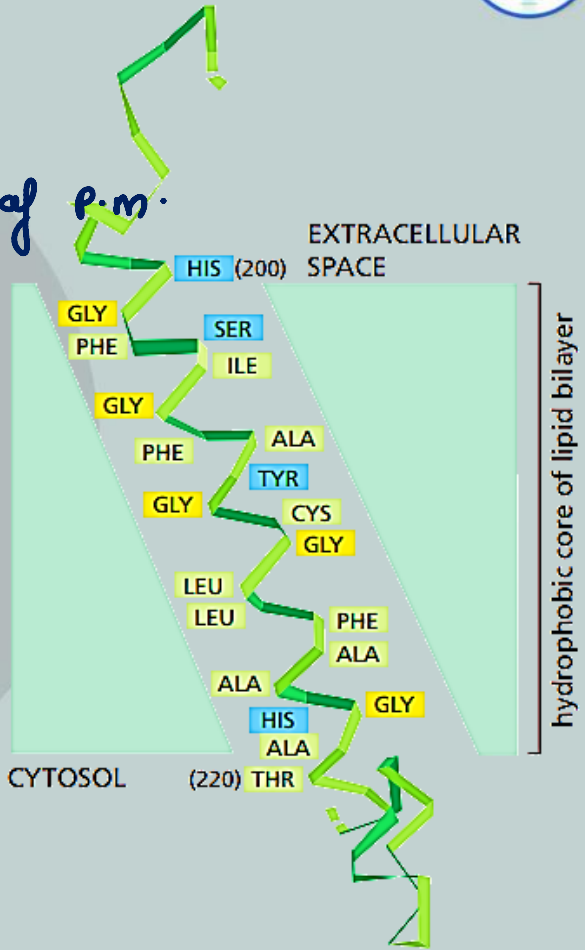
Integral-Transmembrane protein

Presence of α- helices 19-20 hydrophobic residues

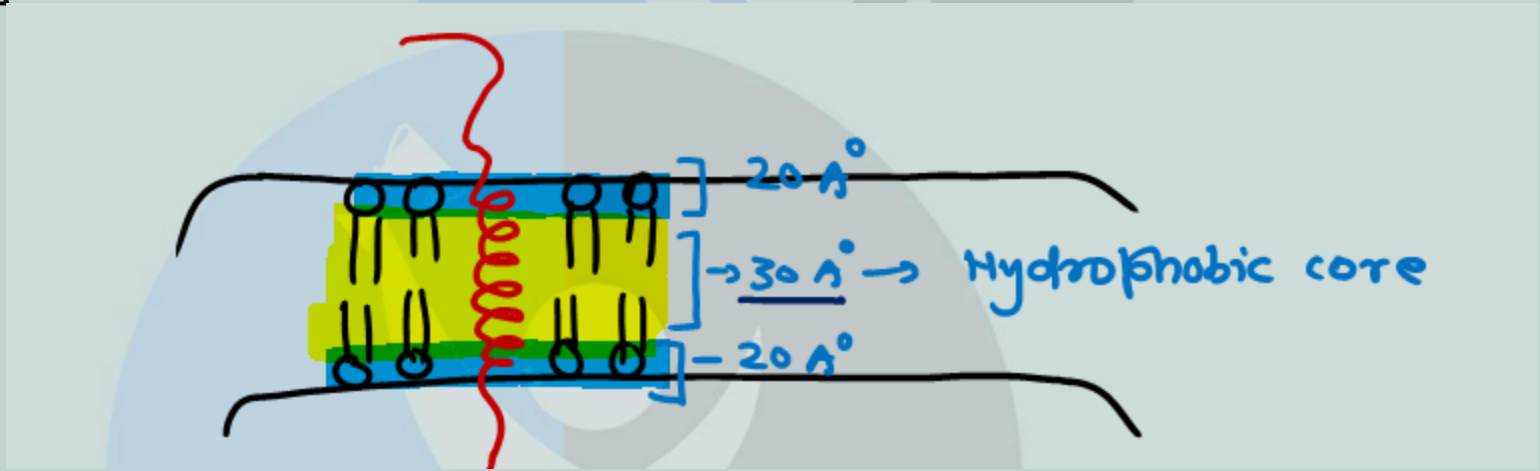
• To Span Hydrophobic core of P.M.

In most transmembrane proteins, the polypeptide chain crosses the lipid bilayer in an α-helical conformation

single-pass transmembrane proteins, the polypeptide chain crosses only once whereas in **multipass transmembrane** proteins, the polypeptide chain crosses multiple times

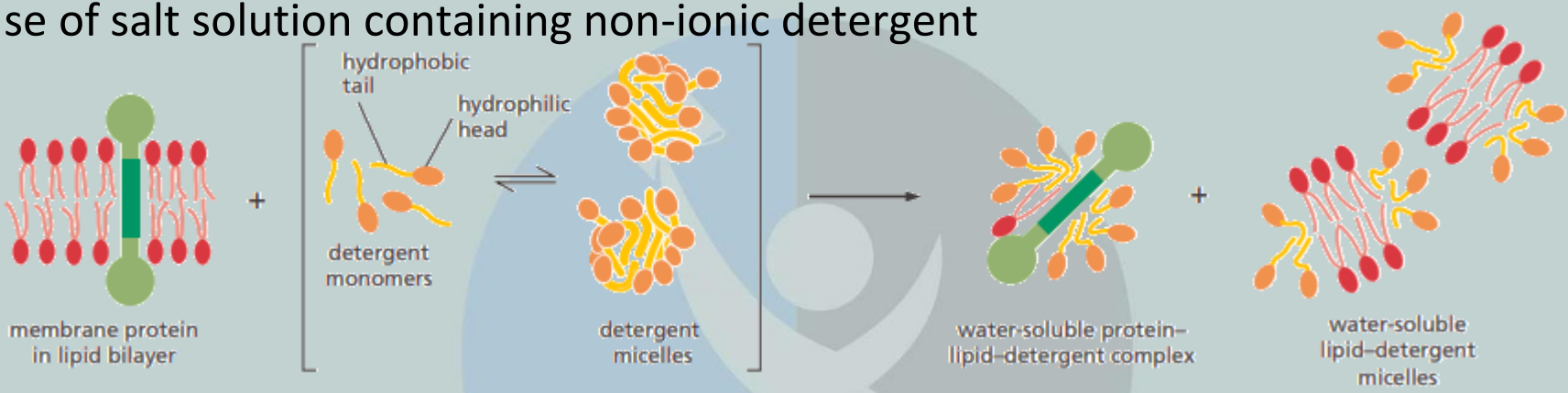


- in alpha Helix - distance between & Amino acid is 1.5 A°
- amino acid - required to cross 30A°→ 30/1.5 = 20 amino acid
- Hydrophobic amino acid required to Cross Pm. at least once 20 A°



Isolation of Integral membrane protein

Use of salt solution containing non-ionic detergent



Use of mild Detergent-

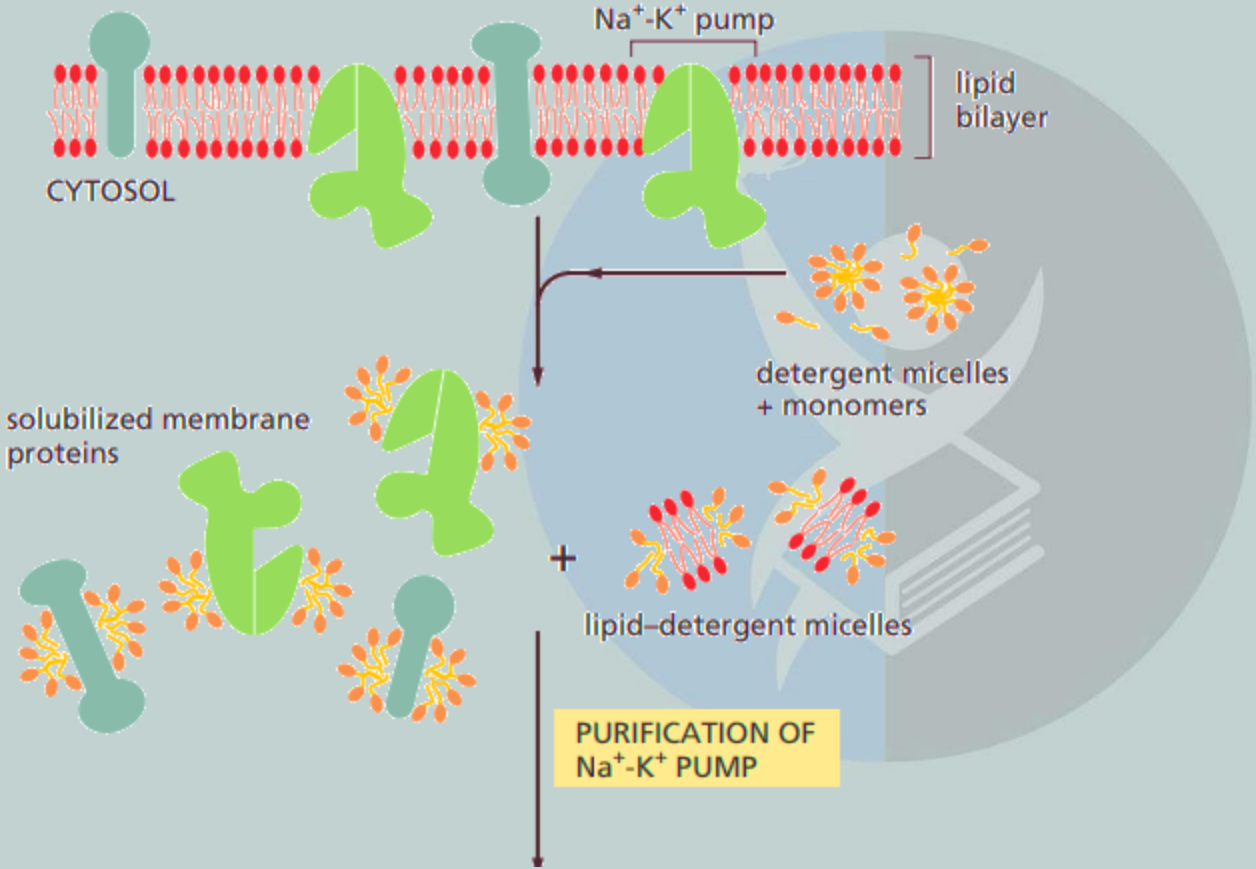
ionic Detergent

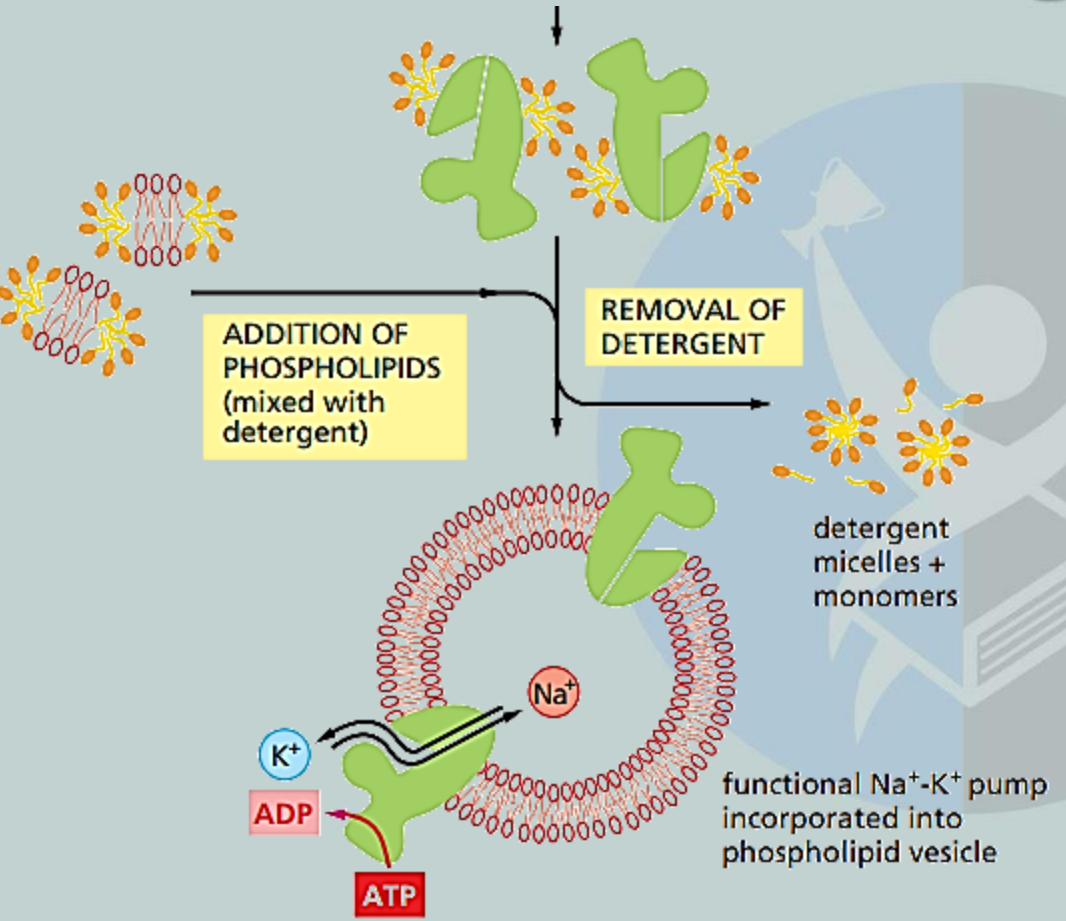
- SDS - -ve
- CTAB- +ve

CTAB

- Triton X

Lipid Raft are resistant to non-ionic detergent

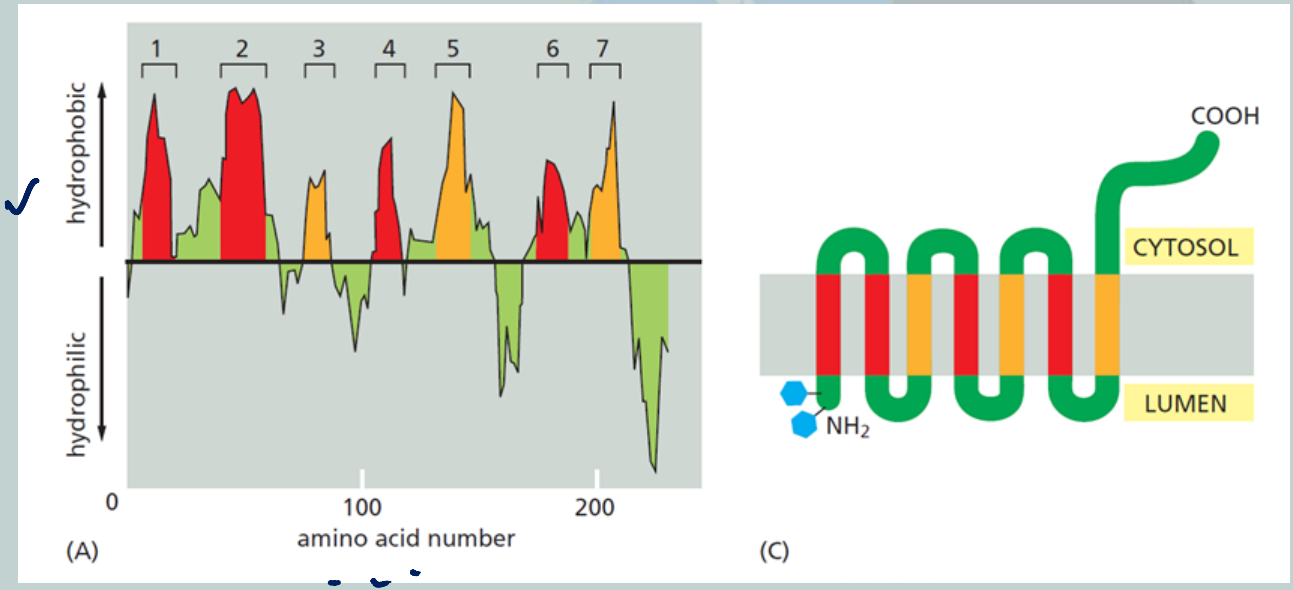

Na+-K+ pump
lipid bilayer
CYTOSOL
detergent micelles + monomers
solubilized membrane proteins
+
lipid–detergent micelles
PURIFICATION OF Na+-K+ PUMP



- The use of mild nonionic detergents for solubilizing, purifying, and reconstituting functional membrane protein systems. In this example, functional Na+-K+ pump molecules are purified and incorporated into phospholipid vesicles. This pump is present in the plasma membrane of most animal cells, where it uses the energy of ATP hydrolysis to pump Na+ out of the cell and K+

Hydropathy Plot

- The hydropathy plot can be used to identify regions of a protein that are likely to be membrane-spanning



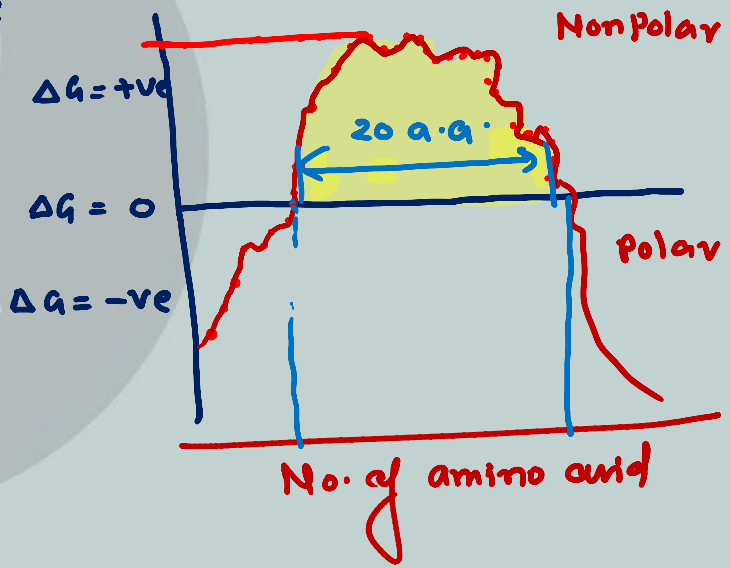
Hydropathy Plot

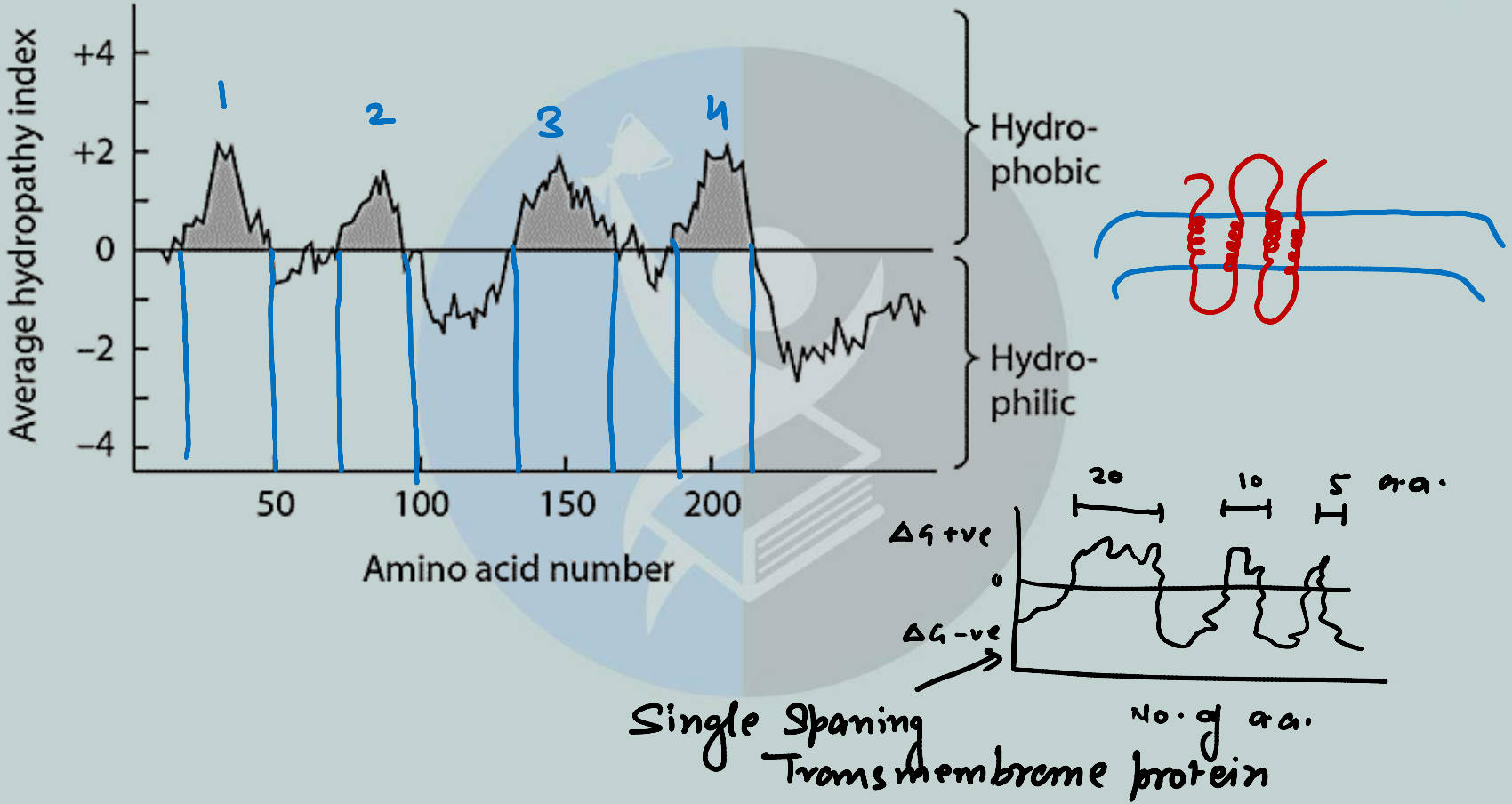
- Arrangement of amino acids on a graph on the basis of their hydrophobicity

- Spontanious Reaction = ΔG = -ve
- Non Spontanious Reaction = ΔG = +ve

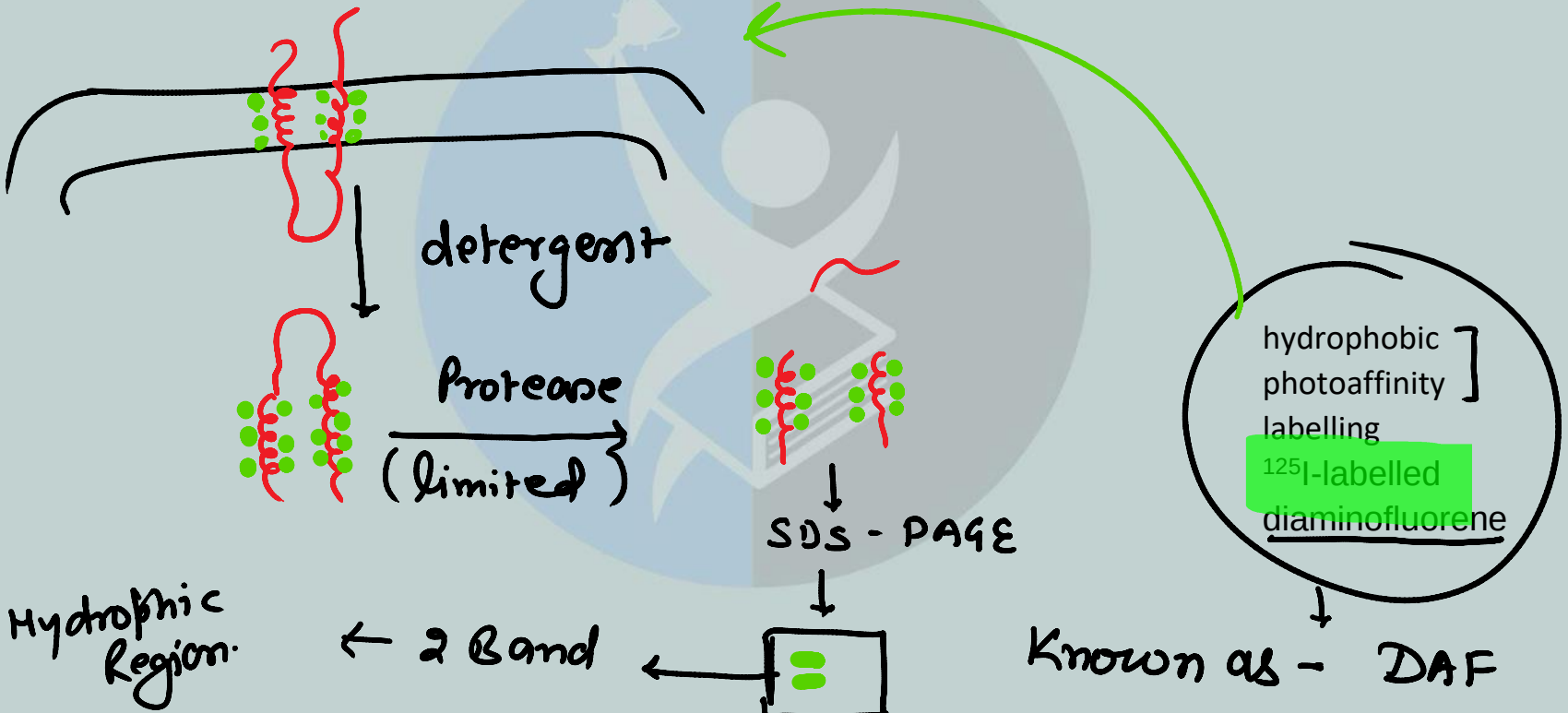
Polar a.a ⟶ ΔG = -ve

Non Polar a.a. ⟶ ΔG = +ve




Average hydropathy index
+4
+2
0
−2
−4
1
2
3
4
50
100
150
200
Amino acid number
Hydro-phobic
Hydro-philic
20
10
5
a.a.
ΔG +ve
0
ΔG -ve
No. of a.a.
Single Spaning
Transmembrane protein

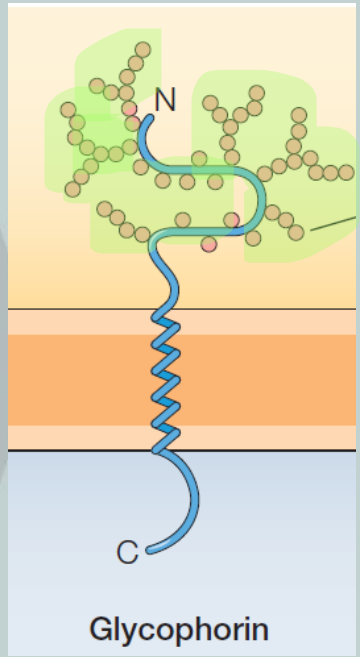
How membrane spanning domain of any integral membrane protein is labelled?



Examples of integral membrane protein in RBC

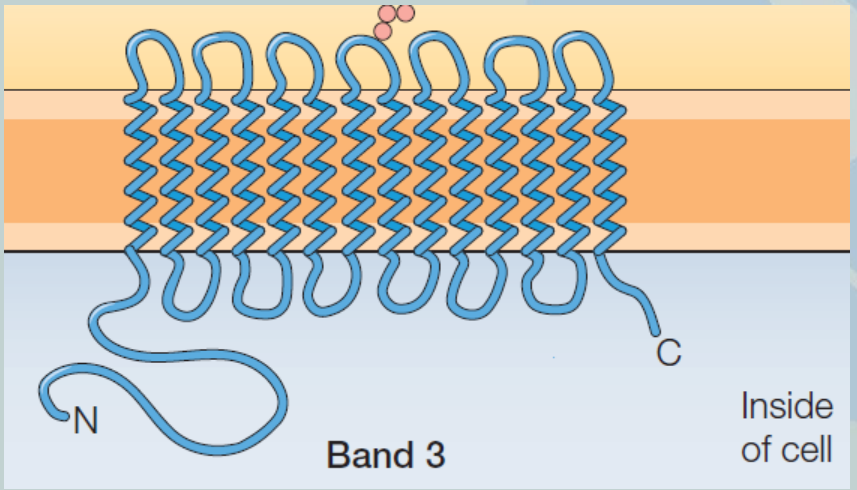
Glycophorin

- Small glycoprotein of 131 amino acids,
- Molecular weight of about 30Kd
- A single membrane-spanning α helix of 23 amino acids.
- N terminus is outside and heavily glycosylated.



Band 3 protein is the anionic transporter

- Bicarbonate (HCO_3^-) and chloride (Cl^-) ions
- 929 amino acids with 14 membrane-spanning α-helical regions.
- Globular structures containing internal channels through which ions are able to travel across the lipid bilayer.
- A lysine group binds anion ((Cl^-))



- **Bacteriorhodopsin** has seven membrane-spanning α helices

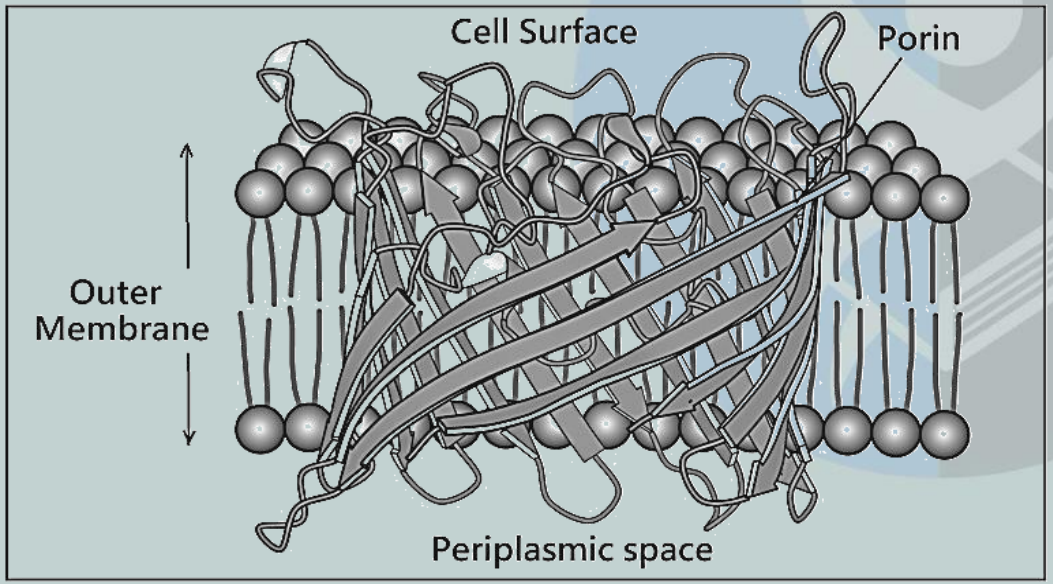
Act as proton pump

- GPCR [G Protein Coupled Receptor]
  - ↳ 7 membrane Spanning α Helix

Most of transmembrane proteins are alpha helix in nature

Porin protein [Exception]

2° Str. → β-Sheet



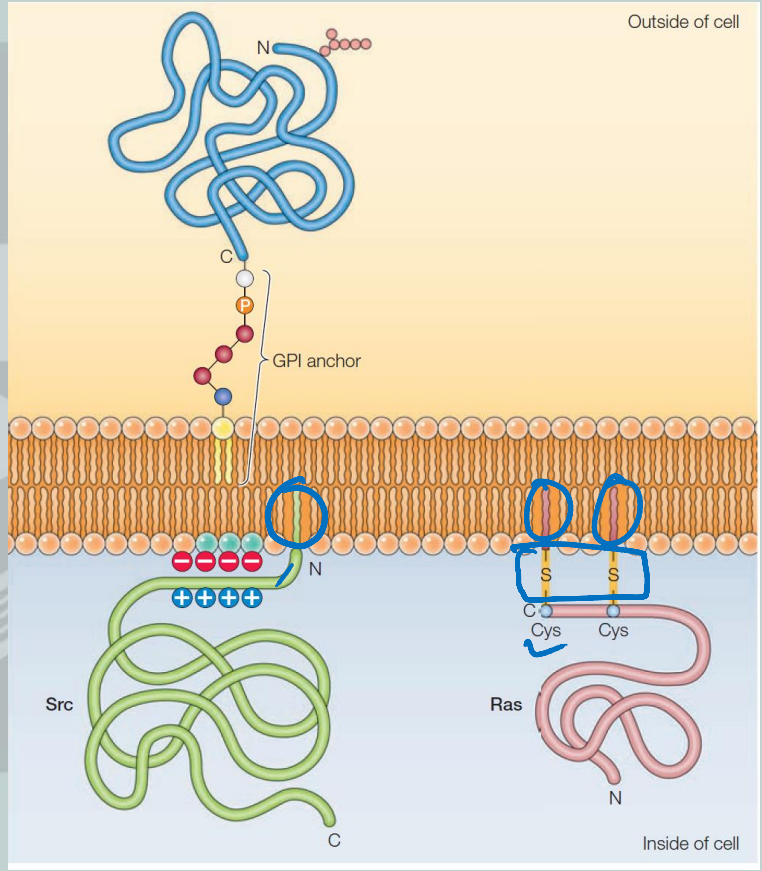
multipass membrane protein structures to be determined by x-ray crystallography. The number of β strands in a β barrel varies widely, from as few as 8 strands to as many as 22

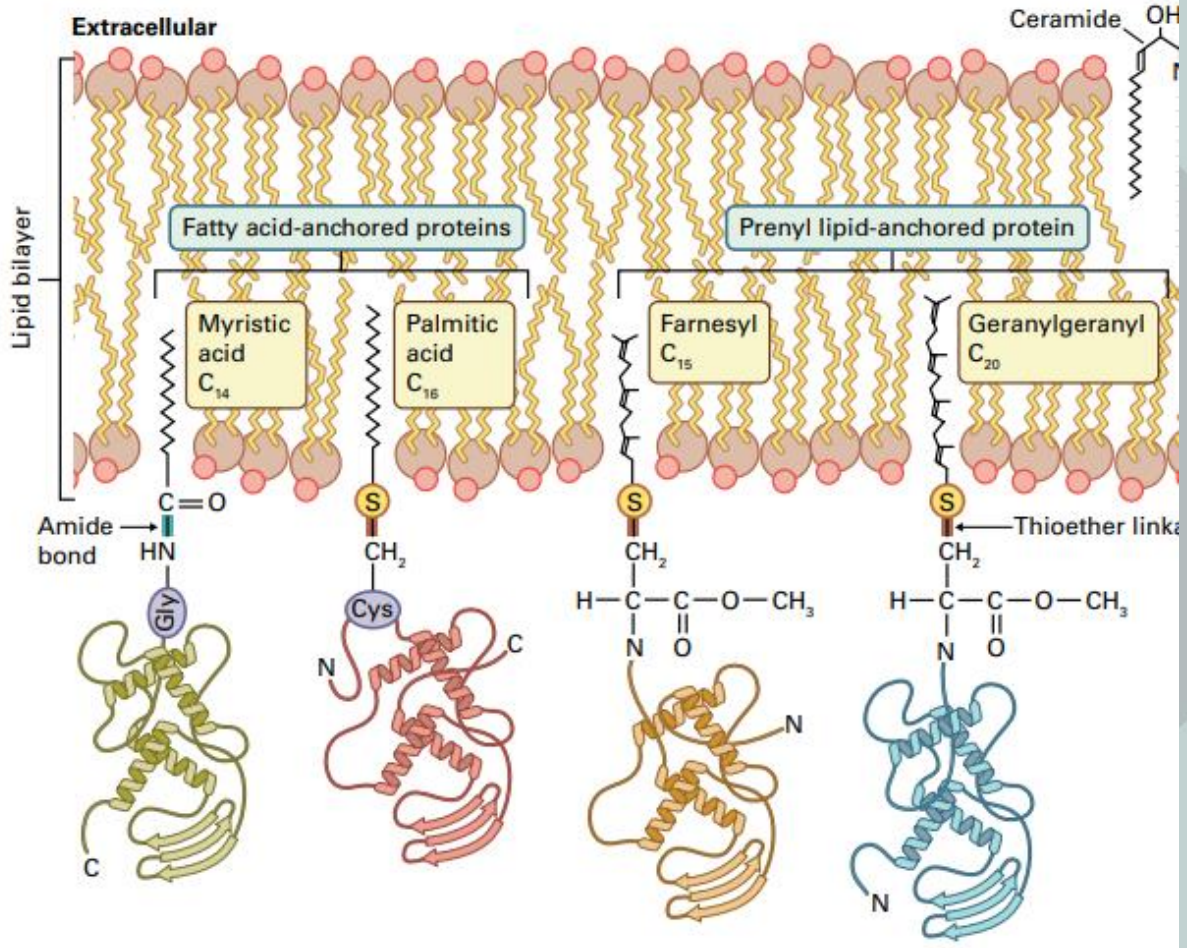
β-barrel proteins are abundant in the outer membranes of bacteria, mitochondria, and chloroplasts. Some are pore-forming proteins, which create water-flled channels that allow selected small hydrophilic molecules to cross the membrane.

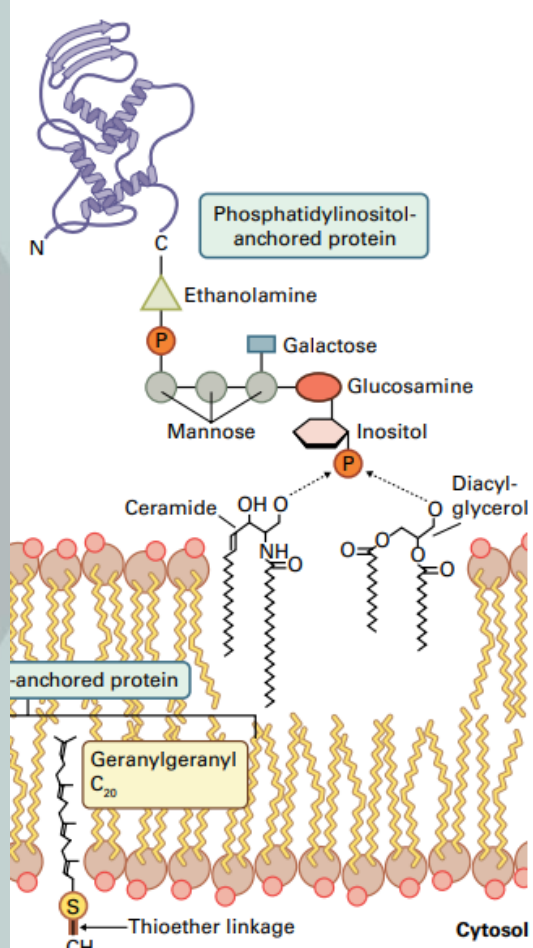
Lipid Anchored Proteins

- Can not removed by salt or detergent treatment
- Protein covalently attached with lipid
- Only lysed by certain enzymes

- Myrsyl Anchored (14 C)
- Prenyl Achored (15 or 20C)
- Palmitoyl Anchored (16 C)
- GPI Anchored Protein



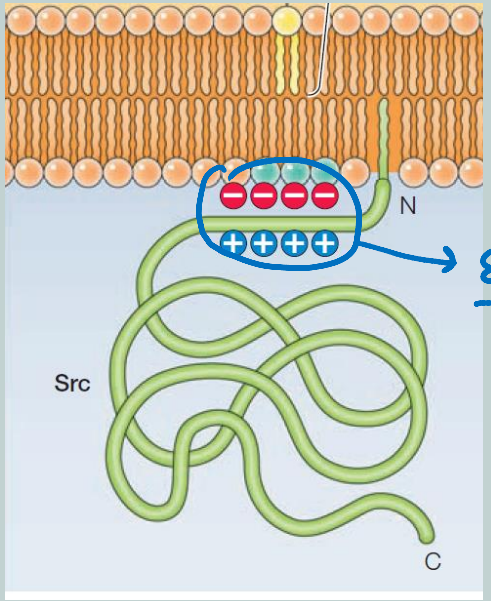

Extracellular
Lipid bilayer
Ceramide
OH
Fatty acid-anchored proteins
Prenyl lipid-anchored protein
Myristic acid C_{14}
Palmitic acid C_{16}
Farnesyl C_{15}
Geranylgeranyl C_{20}
Amide bond
C=O
HN
Gly
S
CH_2
Cys
Thioether linka
N
C


Phosphatidylinositol-anchored protein
N
C
Ethanolamine
P
Galactose
Glucosamine
Mannose
Inositol
Ceramide
OH
O
NH
Diacyl-glycerol
-anchored protein
Geranylgeranyl C_{20}
S
Thioether linkage
Cytosol

Amide-Linked Myristoyl Anchors

Myristic acid linked via an amide bond to the α-amino group of the N-terminal glycine residue of selected proteins.

Eg. α-subunit of *G proteins*

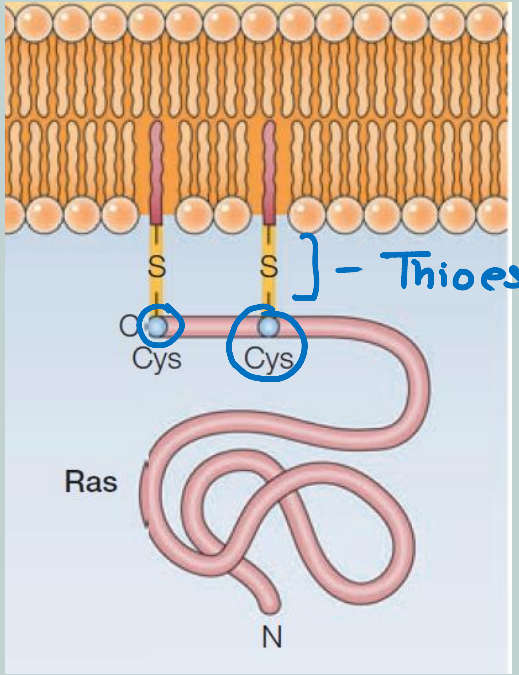


Electrostatic Interaction.

Thioether-Linked Prenyl Anchors

CAAX -- toward c termins

Eg. Ras, Nuclear Lamins



– Thioester bond

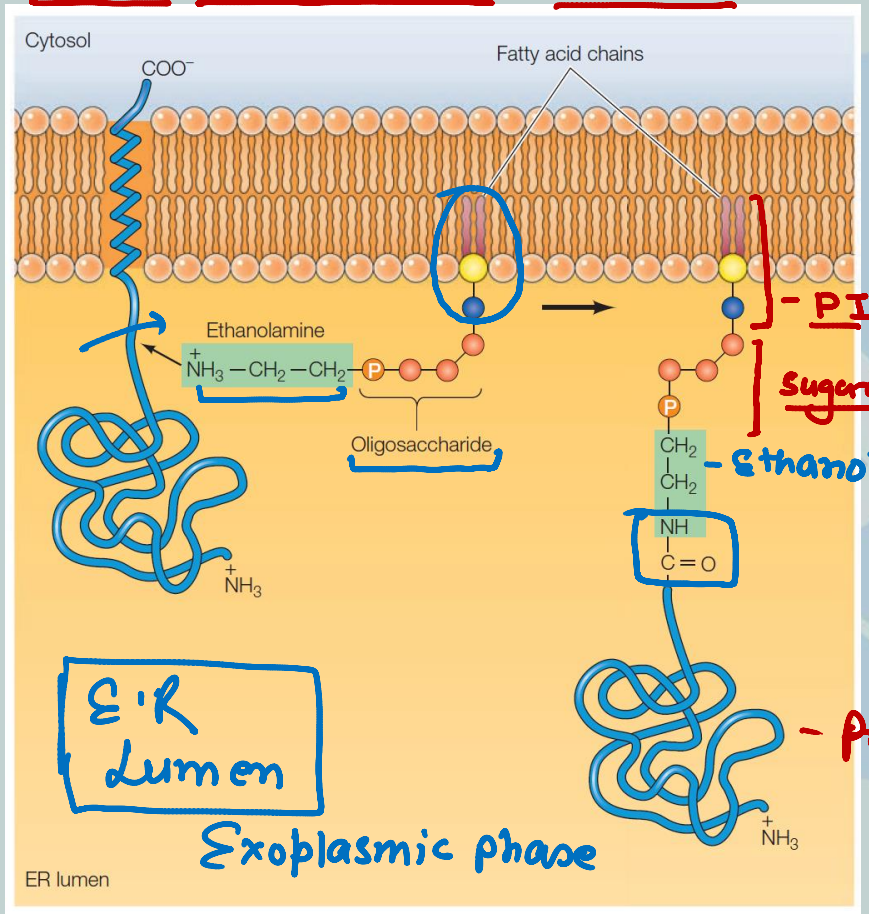
Thioester-Linked Fatty Acyl Anchors

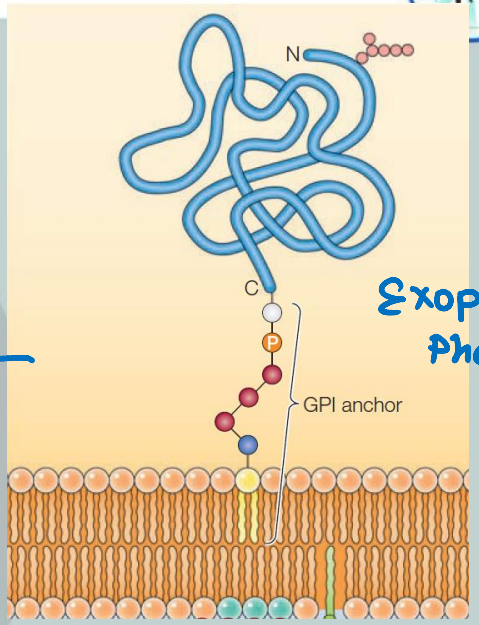
- *Transferrin receptor* protein.

- Modification occurs at c terminus Cys residue

Addition of fatty acyl chain

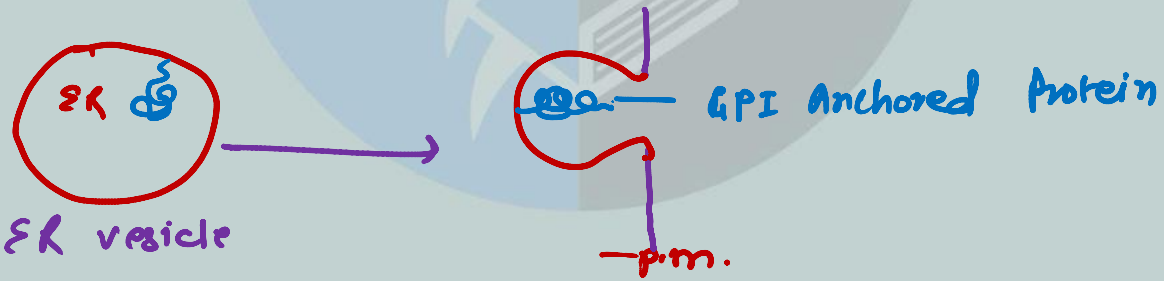
Glycosylphosphatidylinositol (GPI) anchors





Protein is made as single-pass membrane proteins in the endoplasmic reticulum (ER).

While still in the ER, the transmembrane segment of the protein is cleaved of and a glycosylphosphatidylinositol (GPI) anchor is added, leaving the protein bound to the noncytosolic surface of the ER membrane solely by this anchor transport vesicles eventually deliver the protein to the plasma membrane

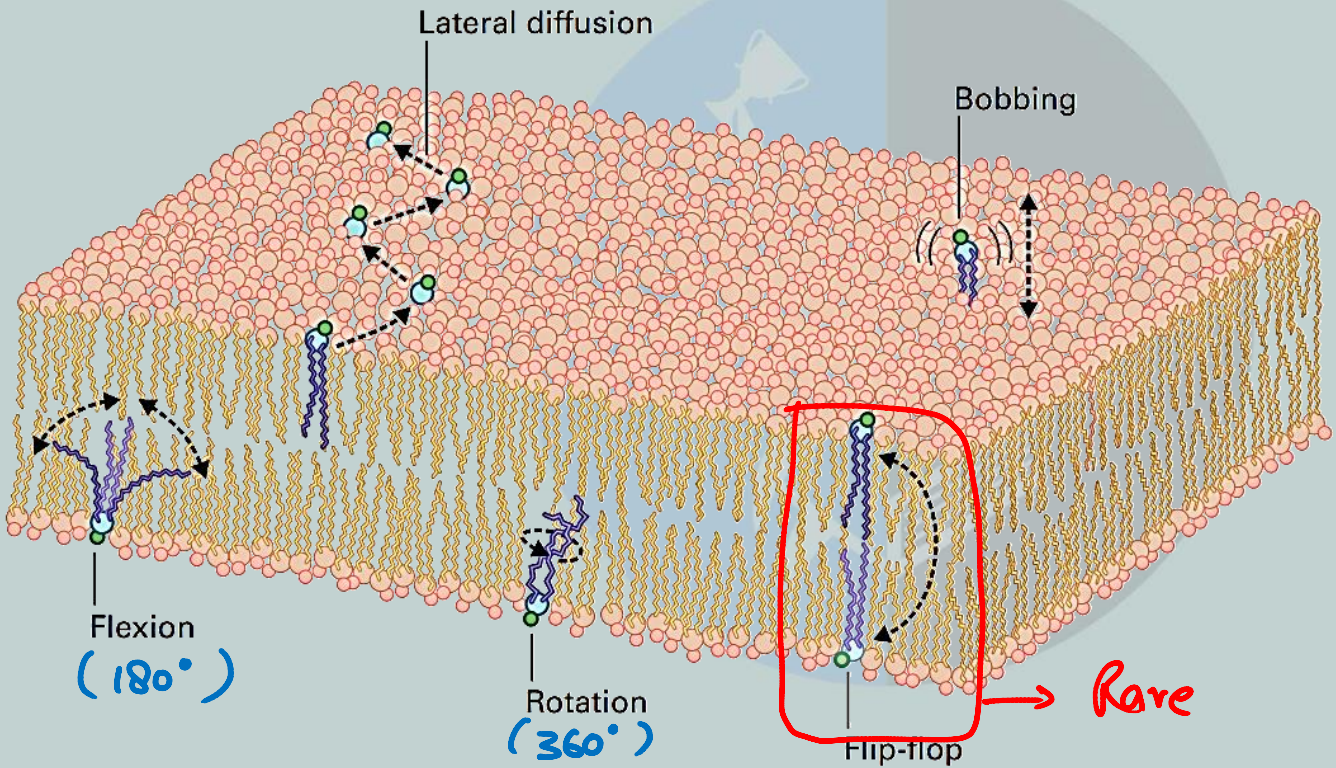


Glycocalyx:

Oligosaccharides of both glycolipids and glycoproteins

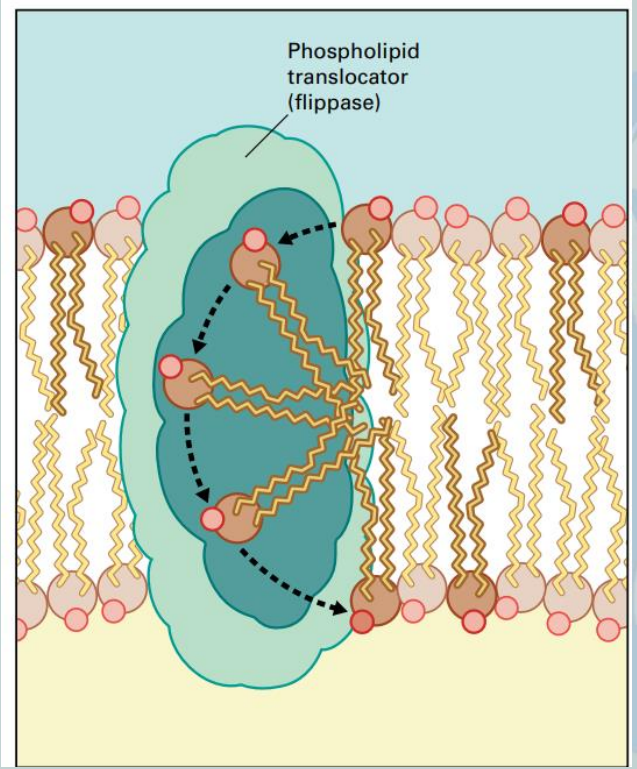
The glycocalyx is a dense, gel-like meshwork that surrounds the cell, constituting a physical barrier for any object to enter the cell. For example, the glycocalyx was identified to play an important role to prevent the entry of pathogens into the cell.

Fluidity of Membrane is due to Lipid Movements



A phospholipid bilayer Phospholipids spontaneously form stable bilayers, with their polar head groups exposed to water and their hydrophobic tails buried in the interior of the membrane.

Individual phospholipids can rotate and move laterally within the bilayer



Mechanism of action of a “flippase,” a phospholipid translocator

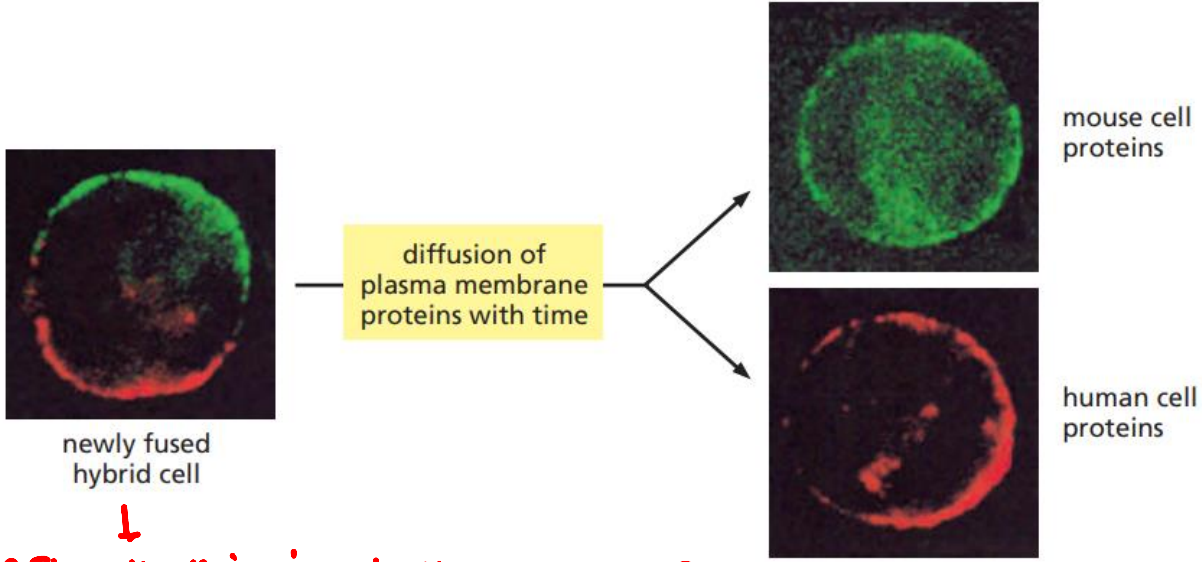
→ movement in lipid molecule

↓

Cause- Fluidity in P.M.

Fluidity of membrane

Cell Fusion experiment: Larry Frye and Michael Edidin in 1970



After 40 min incubation - mix flourescence observed → fluidity is conformed

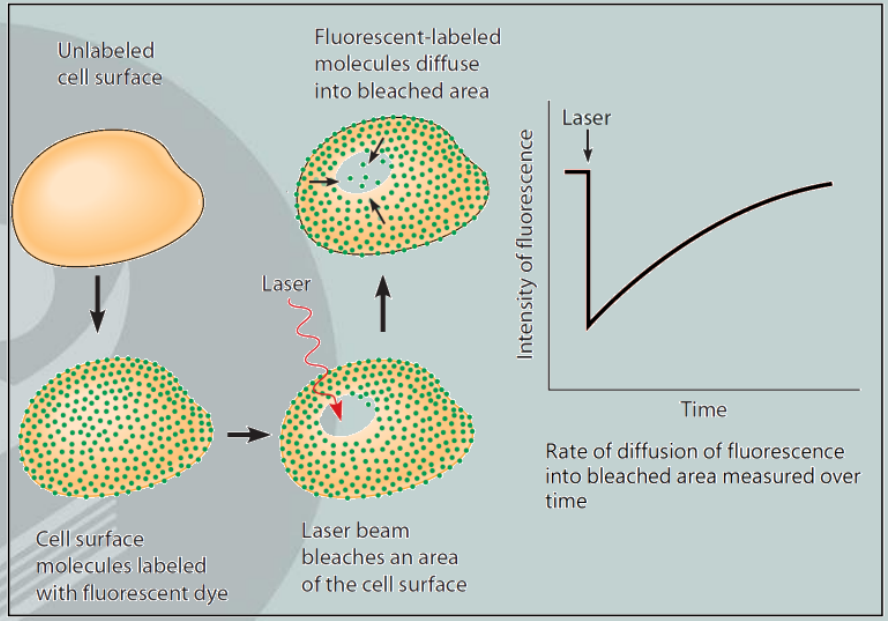
The proteins on the membrane surface are in a dynamic state. ✓

Fluorescence recovery after photo bleaching (FRAP)

The lateral difusion rates of membrane proteins can be measured by using the technique of fuorescence recovery after photobleaching (FRAP).

Fluorescence recovery after photo bleaching (FRAP)

- FRAP is used for investigation of rate of diffusion of lipids and proteins in plasma membrane
- Simple lipids and peripheral protein shows fast recovery in FRAP
- Multiple membrane spanning show slow recovery while cytoskeleton anchored protein shows least recovery.

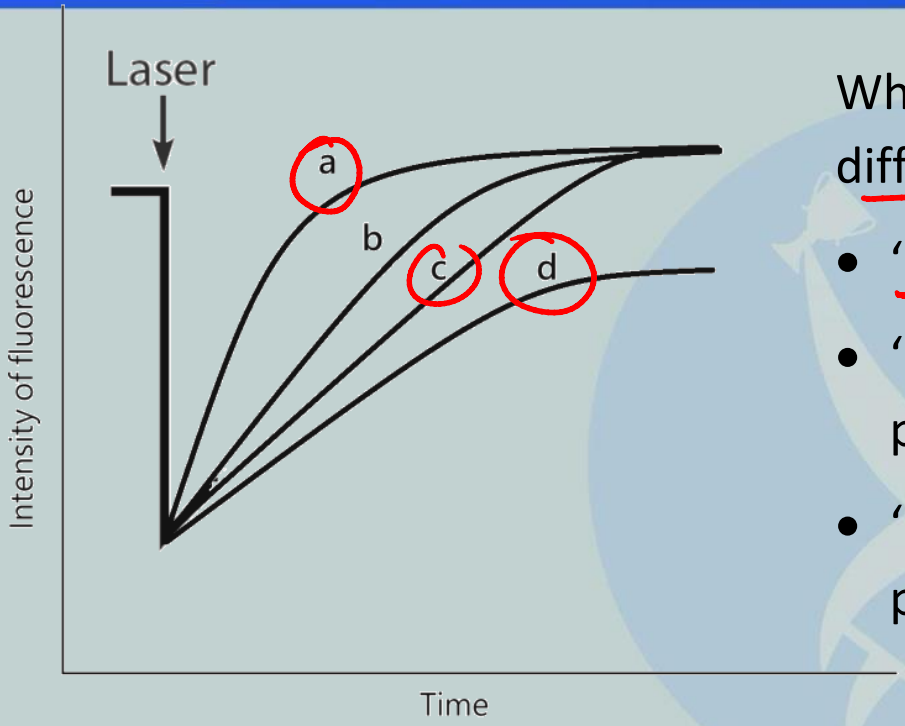

Unlabeled cell surface
Fluorescent-labeled molecules diffuse into bleached area
Laser
Cell surface molecules labeled with fluorescent dye
Laser beam bleaches an area of the cell surface
Intensity of fluorescence
Laser
Time
Rate of diffusion of fluorescence into bleached area measured over time

One drawback to the FRAP technique is that it monitors the movement of large populations of molecules in a relatively large area of membrane; one cannot follow individual protein molecules

Single-particle tracking techniques overcome this problem by labeling individual membrane molecules with antibodies coupled to fuorescent dyes or tiny gold particles and tracking their movement by video microscopy.

Using single-particle tracking, one can record the difusion path of a single membrane protein molecule over time.

Results from all of these techniques indicate that plasma membrane proteins difer widely in their difusion characteristics

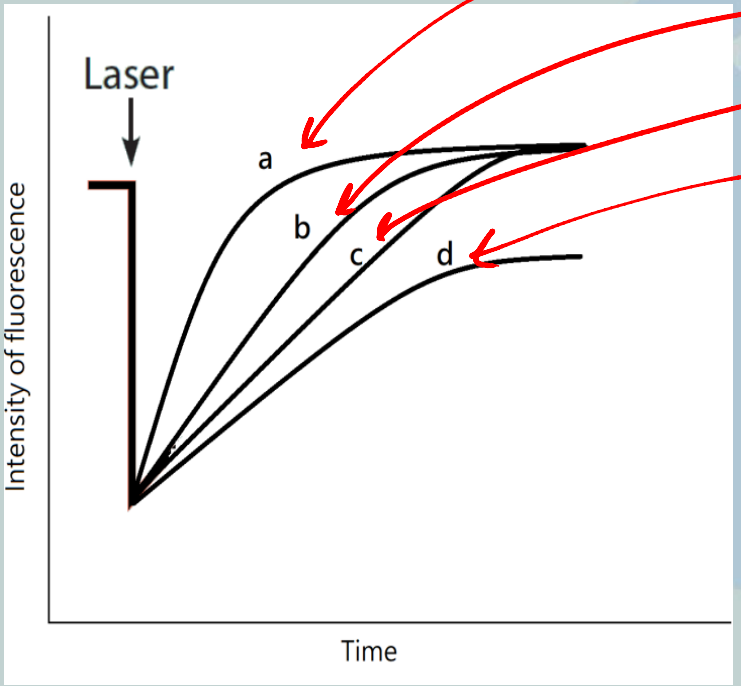


Where, 'a' – is a fluourscent tagged lipid diffusing with fastest rate

- 'b' – is a GFP tagged peripheral protein
- 'c' – is a GFP tagged transmembrane protein
- 'd' – is a GFP tagged transmembrane protein anchored with cytoskeleton

FRAP →

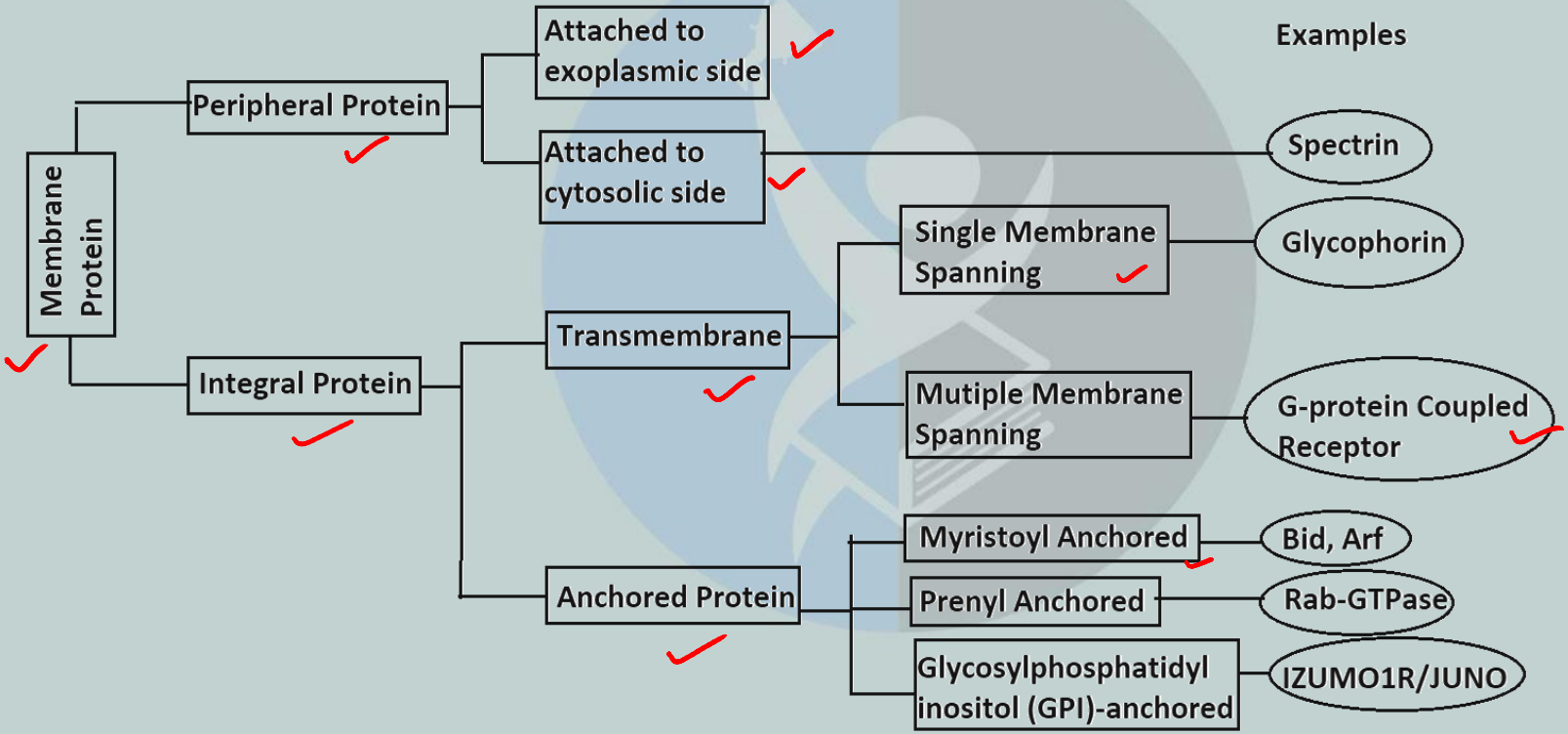
Apply Your Mind



Match with a-d

1. labelled lipid diffusing with
2. labelled Peripheral protein
3. Labelled Integral Protein
4. Labelled Integral protein anchored to cytoskeleton.

Membrane Proteins



www.ifasonline.com

thank you

Learn From India's Best Educators

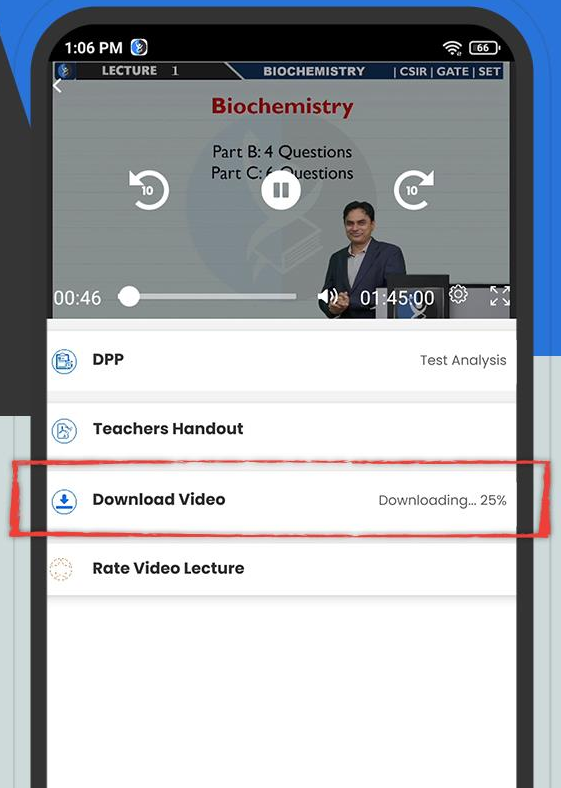
India's **No.1** EdTech Company for Graduates
& Post Graduates Examination.

Download **IFAS** App Now

ANDROID IOS WINDOWS

SAVE YOUR INTERNET DATA USAGE!

Minimize Internet Usage by Downloading Lectures from the IFAS App & Access them Offline



IfAS

Harshada Sharma

Earned: ₹25,500

"As is widely recognized, IFAS stands as India's leading institute for CSIR NET examination preparation. My Personal philosophy has always revolved around helping others and I've had the pleasure of introducing the IFAS Online Course to 12 of my classmates and juniors, which in turn allowed me to earn 25,500 rupees. You too can join in and refer your friends, allowing you to earn a 5% rewards based on the fees each of your referred friends Pay"

REFER & EARN

Step – 01 Invite Friends

Share your referral code or Link with your friends.

Step – 02 Earn Cashback

On Every Purchase made using your referral code, you earn 5% instant cashback in your bank account.

Step – 03 Refer More, Earn More

Keep referring as many friends as you can and keep **Earning** cash as much as you can

Friendship is Sharing, Caring, Giving & Helping Each Other...!